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Comparative Fecundity During Laboratory Rearing in South Carolina (March-May) of Field-Collected Versus F1 Laboratory-Reared Laricobius nigrinus (Coleoptera: Derodontidae)

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COMPARATIVE FECUNDITY DURING LABORATORY REARING IN SOUTH CAROLINA (MARCH-MAY) OF FIELD-COLLECTED VERSUS F1 LABORATORY-REARED *LARICOBIUS NIGRINUS* (COLEOPTERA: DERODONTIDAE)

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ABSTRACT

The purpose of this study was to determine whether there is a difference in *Laricobius nigrinus* Fender fecundity between field-collected and laboratory-reared beetles. *Laricobius nigrinus* used for this study were separated into four cohorts based on origin: Cohort 1 – Wild-caught from Seattle, WA – native population; Cohort 2 – Wild-caught from Banner Elk, NC – naturalized population; Cohort 3 – Laboratory-reared F_1 generation from Seattle, WA parents; and Cohort 4 – Laboratory-reared F_1 generation from Banner Elk, NC parents. In 2013, the first year of this study, gender determination of live beetles had not been developed for *L. nigrinus.* For that reason, the study was set up following the mass-rearing protocols for *L. nigrinus*. Due to the scale of the study in 2013, the study yielded only one repetition per cohort. In 2014, new protocols allowed determination of gender of live adults. That allowed me to reduce the scale of the study allowing me to conduct a more detailed replicated study.

In 2013, the PNW Cohort yielded fewer live larvae and total (live plus dead) larvae than any other cohort, but produced the highest number of larvae per female. This cohort had a highly skewed male to female ratio with far fewer females compared to males. This is the most likely cause of the low larval production for the PNW Cohort in 2013. In 2014, analyses of the least squares means for the parameters: larvae per female, live larvae, total larvae, and eggs were all significantly greater for field-collected cohorts compared to their respective laboratory-reared F_1 offspring. My study confirms that wild-caught cohorts produced a higher number of larvae per female than laboratory-reared cohorts.

Variables other than laboratory-rearing, such as *L. nigrinus* x *L. rubidus* hybridization and food source differences (*i.e., A. tsugae* on eastern vs western hemlock), are potential contributors to the observed decrease in larvae per female for wild-caught versus F_1 cohorts. I recommend further study be conducted on these variables. I also encourage further research into the development of field-insectaries for mass-rearing *L. nigrinus.*

DEDICATION

To my parents, Željko and Ljuba Trninić. Thank you for your unyielding support and invaluable guidance, and for always reminding me of the things that truly matter in life. I love you both.

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CHAPTER ONE

INTRODUCTION

Adelges tsugae Annand [Hemlock Woolly Adelgid; Hemiptera: Adelgidae] is found naturally on hemlock (*Tsuga*) trees in Eastern Asia and the North American Pacific Northwest. However, since it was accidentally introduced into the Eastern United States from Japan in the early 1950s, it has become a major destructive force to *Tsuga* species in its nonnative habitat (Havill et al., 2006, Havill & Montgomery, 2008, McClure & Cheah, 1999, Stoetzel, 2002). The Eastern U.S. is home to two native species of hemlock, *Tsuga canadensis* (L.) Carrière (eastern hemlock) and *Tsuga caroliniana* Engelmann (Carolina hemlock) (Goodman & Lancaster, 1990, Havill & Montgomery, 2008). *Adelges tsugae* feed on the xylem parenchyma cells at the base of hemlock needles. This results in needle loss and prevents new needle formation in *T. canadensis* and *T. caroliniana*, causing widespread mortality in these two species (Mausel et al. 2008, McClure & Cheah, 1999, Shields et al., 1995). In 2012, *A. tsugae* could be found as far west as Kentucky and Tennessee, as far north as Maine and as far south as Georgia, (USDA Forest Service, 2013). In an effort to control the destructive effects of *A. tsugae*, both chemical and biological control methods have been implemented (Fidgen et al., 2002, McClure, 1987). While chemical control has been effective, it becomes an impractical solution on a scale that would effectively control the extent of the infestation in the Eastern U.S. (McClure, 1987).

Laricobius nigrinus Fender (Coleoptera: Derodontidae), a native predator of *A. tsugae* in the Pacific Northwest, has been released in the Eastern U.S. since 2003 (Mausel et al., 2010). *Laricobius nigrinus* is a specialist predator feeding almost exclusively on *A. tsugae*, and while it will consume other insects for sustenance, *L. nigrinus* can only complete its life cycle on *A. tsugae* (Zilahi-Balogh et al., 2002). *Laricobius nigrinus* has been mass-reared for release in several insectaries in the Eastern U.S., and has also been imported from the Pacific Northwest for direct release in *A. tsugae*-infested regions of the Eastern U.S. Depending on the scale of release efforts, rearing *L. nigrinus* in insectaries may involve significant commitments of funding, resources, and manpower, and despite years of effort directed to creating ideal rearing conditions, laboratory-reared beetles become increasingly lethargic and less fecund when maintained in a laboratory (Lamb et al., 2005, Wallin et al., 2011). When compared to wild-caught beetles from the Pacific Northwest, laboratory-reared beetles produce noticeably fewer offspring (Lamb et al., 2005). Additionally, a study by Mausel et al. (2008) examining the use of field insectaries for producing *L. nigrinus* indicated that field-insectary-reared beetles appeared both larger and healthier than laboratory-reared beetles. Conclusions by Mausel et al. (2008) support the idea that field-collected *L. nigrinus* would be more fecund based on Zilahi-Balogh's (2001) finding that there is a positive relationship between fecundity and female size. This would suggest that field-insectary-reared beetles may likely have greater fecundity compared to laboratory-reared beetles.

This study examines claims that fecundity of *L. nigrinus* decreases for beetles that have been kept in a laboratory setting for one generation versus fecundity of *L. nigrinus* that have been brought into a laboratory setting directly from the field. This project will examine the variance in fecundity among four *L. nigrinus* cohorts: Cohort 1 – Wild-caught *L. nigrinus* from Seattle, WA – native population (PNW); Cohort 2 – Wild-caught *L. nigrinus* from Banner Elk, NC – naturalized population (NC); Cohort 3 – Laboratory-reared *L. nigrinus* F_1 generation from Seattle, WA parents (PNW-F_1); and Cohort 4 – Laboratory-reared *L. nigrinus* F_1 generation from Banner Elk, NC parents (NC-F_1).

To determine if fecundity decreases over a single generation of laboratory rearing, I conducted an analysis of *L. nigrinus* fecundity and adult mortality from four cohorts with different origins in 2013 and 2014. The 2013 study provided preliminary data with one repetition per cohort, which allowed the 2014 study to be modified using eight repetitions per cohort to provide more detailed information. My study adds to the knowledge of *L. nigrinus* and addresses the questions of whether there is, 1) a quantifiable difference in fecundity between wild-caught and laboratory-reared *L. nigrinus*, 2) a quantifiable difference between native (WA) and naturalized (NC) populations, 3) do the two wild-caught cohorts display significant differences in fecundity (PNW vs NC) and do the two laboratory-reared cohorts display a significant difference in fecundity (PNW-F_1 vs NC-F_1), and 4) is there a significant difference in female and male mortality among cohorts?

Another concern related to releasing *L. nigrinus* in the Eastern U.S. is that they have been shown to hybridize with the native *L. rubidus* LeConte. Genetic techniques to discern the two species and their hybrids have been developed. Morphological differences including the proportion of black in beetle elytra and the angles of the parameres in male genitalia have been shown to be parameters with significant differences between *L. nigrinus* x *L. rubidus* hybrids versus pure parent strains (Havill et al. 2012). Because the ultimate implications of hybridization at this time are only speculative, further studies are needed to determine the impact of hybridization on *Laricobius* populations in the Eastern U.S. (Havill et al. 2012).

By collecting and analyzing data for each cohort on the number of larvae produced by the number of adult females available, my study provides a quantified understanding of laboratory rearing-induced changes to *L. nigrinus* fecundity.

CHAPTER TWO

LITERATURE REVIEW

Hemlock Trees (*Tsuga*):

Hemlock (*Tsuga* spp.) trees are long-lived gymnosperms found in North America and Asia. Eastern hemlock, *T. canadensis*, and Carolina hemlock, *T. caroliniana*, are native to the Eastern U.S. (Goodman & Lancaster, 1990, Havill & Montgomery, 2008). Eastern hemlock ranges from Maine to Georgia, and westward into Ohio, Michigan and Wisconsin (Fig. 1). Carolina hemlock has a much more limited range being found only in certain counties in South Carolina, Georgia, North Carolina, Virginia, Tennessee, and one county in Ohio (United States Department of Agriculture Natural Resources Conservation Service, n.d., United States Department of Agriculture Forest Service, 2013).

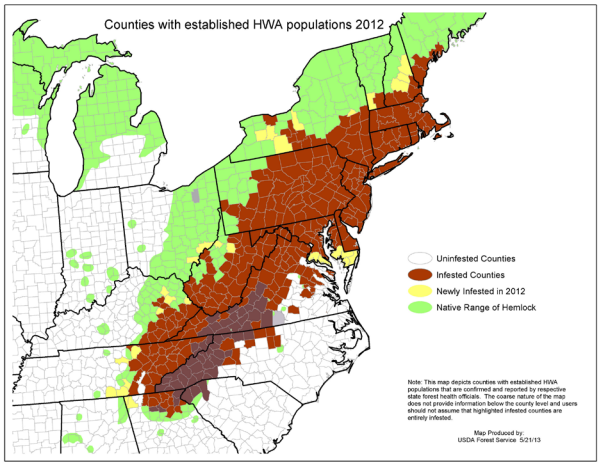

Figure 1: USDA Forest Service 2012 map showing the range of native Eastern hemlock without *A. tsugae* infestations in green and the range of Carolina hemlocks without *A. tsugae* infestations in light gray. *Adelges tsugae* infested counties with eastern hemlock only are shaded red, while those with both eastern and Carolina hemlocks are shaded dark gray. Newly infested counties as of 2012 are shaded yellow (United States Department of Agriculture Natural Resources Conservation Service, n.d., United States Department of Agriculture Forest Service, 2013).

Hemlock trees are an integral part of forest ecosystems of the Eastern U.S. Hemlocks require moist soil and are often found in or near coves and riparian zones (Goodman & Lancaster, 1990, Martin & Goebel, 2012). Hemlock presence has a direct effect on both terrestrial and aquatic flora and fauna (Eschtruth et al., 2006, Ross et al., 2003) as these trees provide food, shelter, and environmental conditions supporting wildlife species including deer, birds, fish, salamanders and land and aquatic invertebrates. (Dilling et al., 2007, Havill et al. 2014, Reay, 2000, Tingley et al., 2002). Due to the infestation of *A. tsugae*, hemlock trees and the ecosystems that they inhabit are under serious threat (McClure & Cheah, 1999, Havill & Montgomery, 2008).

***Adelges tsugae* (Hemlock Woolly Adelgid):**

Adelges tsugae is native to Eastern Asia and the Pacific Northwest (Havill et al., 2006, Cheah et al., 2004). *Adelges tsugae* has a polymorphic life cycle, with two generations per year observed in the Eastern U.S. The short-lived progrediens generation emerges in the late winter and early spring. It includes both asexual winged (sexuparae) and wingless forms. The wingless progrediens continue inhabiting *Tsuga*, while in parts of Asia the winged sexuparae disperse onto suitable spruce species (*Picea*) and produce a short-lived sexual (males and females) generation (sexuales). Mated females produce a single asexual female (fundatrix), which in turn produces multiple winged asexual females (gallicolae) that mature on spruce before returning to hemlock to complete their life cycles (Havill & Foottit, 2007, Havill et al., 2014, McClure, 1996). In North America there are no suitable spruce species for *A. tsugae*, which results in the death of sexuparae

(Cheah et al., 2004). In late spring, eggs produced by the asexual progrediens give rise to the completely asexual, long-lived sistens generation. Sistens emerge as crawlers in the early summer from ovisacs on hemlock, locate a feeding site then aestivate through the warmest part of the summer. In mid-Autumn, sistens break aestivation and begin feeding. By mid-winter, they complete their four instars and reach adulthood. Adults produce eggs, which give rise to progrediens in late winter and early spring (McClure, 1987). Sistens are wingless and disperse primarily by phoresis and wind.

From the second instar through the adult stage, sistens produce a wool-like coating from lipid-based (waxy) filamentous material secreted from pores on their bodies. As adults, females lay their eggs within woolly ovisacs, which provide protection from predators and abiotic factors (McClure, 1987).

Adelges tsugae was most likely unintentionally introduced into the Eastern U.S. from an ornamental hemlock tree imported from Japan to a Virginia nursery in 1951 (Havill & Montgomery, 2008, Stoetzel, 2002). From there, the population was transported by wind, migratory birds, and vertebrates including humans, onto native hemlocks throughout the Eastern U.S. (McClure & Cheah, 1999). Because *T. canadensis* and *T. caroliniana* evolved separately from *A. tsugae*, these species display no inherent resistance to the insect nor does their environment contain effective *A. tsugae* predators, pathogens, or parasitoids, leaving these two native species highly vulnerable to the introduced adelgid (Cheah et al., 2004).

Adelges tsugae feeds on *Tsuga* by inserting its piercing/sucking mouthparts into the parenchyma cell at the base of a needle (McClure & Cheah, 1999, Young et al., 1995). *Adelges tsugae* damage can be detected initially by a discoloring of the hemlock needles. Trees are eventually killed due to defoliation (McClure, 1987). In the Eastern U.S. *A. tsugae*-induced hemlock mortality can occur in as little as one year, although infested trees may survive for a decade or longer (McClure, 1987, Webb et al., 2003). In its native environment *A. tsugae* does not have any significant impact on native hemlocks due to the occurrence of coevolved biological control species and potential chemical resistance by hemlock species native to Asia and the Pacific Northwest (Cheah et al., 2004). In the Eastern U.S., few forest ecosystems have been left undamaged by this introduced pest (Cheah et al., 2004).

Controlling *A. tsugae*:

In an attempt to control the infestation of *A. tsugae* in the Eastern U.S., both chemical and biological control methods have been implemented (Fidgen et al., 2002, McClure, 1987). Pesticides containing imidacloprid, dinotefuran, or thiamethoxam applied as soil drenches, soil injections, trunk sprays, or trunk injections have all proven effective for management of *A. tsugae* after they have begun feeding and producing the wool covering (Fidgen et al., 2002, Webb et al., 2003). Although pesticides have shown success, chemical control is an impractical solution over the landscape scale distribution of hemlocks. Because these trees are often found in riparian ecosystems, there is also the

potential for contamination of streams and lakes. Chemical controls could also lead to secondary infestations of other pest species such as scale or spider mites (McClure, 1989).

Biological control via generalist and specialist predators has been heavily researched and implemented in an effort to manage the infestation of *A. tsugae* at the landscape-scale on a long-term basis. Among biological control agents, *Sasajiscymnus tsugae* Annand [Coleoptera: Coccinellidae]), *Scymnus* (*Neopullus*) (Coleoptera: Coccinellidae) (*Scymnus sinuanodulus* Yu et Yao and *Scymnus ningshanensis* Yu et Yao), *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), and *Laricobius osakensis* Montgomery and Shiyake sp. nov. have been the focus of rearing and release for biological control of *A. tsugae* in the Eastern U.S. (Cheah et al., 2004, McClure, 1987).

***Laricobius nigrinus*:**

Laricobius is one of four genera in the family Derodontidae (LeConte), and while the other genera all feed on fungus, *Laricobius* is predatory on *Adelgidae* (Lawrence, 1989). *Laricobius nigrinus* is a specialist predator of *A. tsugae* native to the Pacific Northwest. In the Pacific Northwest, *A. tsugae* feed on western hemlock (*Tsuga heterophylla* (Rafinesque) Sargent) and mountain hemlock (*Tsuga mertensiana* (Bongard) Carrière), which range from Alaska south to California and east to Montana (Cheah et al., 2004, Taylor, 1993). Research on *L. nigrinus* as a biological control agent for *A. tsugae* began in 1997 when these beetles were first imported to the quarantine facility at Virginia

Polytechnic Institute and State University for study after they had been observed as predators of *A. tsugae* in British Columbia (Cheah et al., 2004). Since that time, *L. nigrinus* has been extensively studied as a biological control agent of *A. tsugae*.

Although *L. nigrinus* produces only one generation per year, its life cycle closely resembles that of *A. tsugae,* its primary prey, being active in winter and aestivating during summer. *Laricobius nigrinus* eggs are bright yellow and are oviposited into the wool around female *A. tsugae.* While *L. nigrinus* can survive by feeding on other small soft-bodied insects, it can only complete its life cycle when feeding on *A. tsugae* (Zilahi-Balogh et al., 2002). Although *L. nigrinus* feeds on all stages of *A. tsugae*, larvae feed almost exclusively on eggs. Once mature, larvae of *L. nigrinus* drop to the ground and burrow into the soil where they pupate. Adults emerge in the soil where they aestivate until autumn before emerging (Cheah et al., 2004, Mausel et al., 2010, Zilahi-Balogh et al., 2003).

Rearing *L. nigrinus* in the laboratory can be difficult, as the environmental requirements for development change for different life stages. During the pre-oviposition/laboratory-storage stage of their life cycle from autumn to late winter/early spring, *L. nigrinus* are kept at 4^{o}C and a 10:14 light:dark cycle. During oviposition in late winter and spring adults are kept at 8^{o}C and a 12:12 light:dark cycle. Larvae emerge from late winter to early summer and are kept at 13^{o}C and a 12:12 light:dark cycle. In the spring and summer, mature larvae are placed inside containers with a sphagnum peat moss and sand

mixture (referred to as soil in the rest of the manuscript) where they pupate for approximately 14 days then remain in aestivation as adults through the summer. Once mature larvae are placed in soil, they are kept at 13^{o}C and a 12:12 light:dark cycle for 41 days before they are transferred to an aestivation chamber and kept at 19^{o}C and a 16:8 light:dark cycle until autumn. In autumn, aestivation containers are kept at 13^{o}C and a 8:16 light:dark cycle where they begin to emerge as adults. At this point, *L. nigrinus* are put into storage and the cycle begins anew. During all stages, *Laricobius nigrinus* are kept at 60%-65% humidity. During pupation and aestivation, soil moisture is kept at approximately 30% (Lamb et al., 2007, Salom et al., 2012). Both adults and immature larvae require a constant supply of healthy *A. tsugae* on which to feed (Cheah et al., 2004). Hemlock twigs infested with *A. tsugae* were field-collected in South Carolina and Georgia for this study.

Rearing *L. nigrinus* in an insectary appears to lead to decreased fecundity beginning with the F_1 generation. To remedy this problem, one suggestion for mass rearing this species is to have an insectary begin the annual cycle with an initial colony of ≥2000 *L. nigrinus* individuals when using laboratory-reared adults, versus only 500 to 1000 adults if field-collected *L. nigrinus* are available (Lamb et al., 2005). This indicates that fecundity of laboratory-reared adults may be as little as one-half to one-quarter that of field-collected individuals.

Factors other than fecundity seem to be affected by laboratory rearing of *L. nigrinus.* Wallin et al. (2011) found that in olfactometer bioassay tests, *L. nigrinus* had varying responses to odors from host trees infested with *A. tsugae*, but did not respond to odors from *A. tsugae* alone. They suggested that *L. nigrinus* was responding to hemlock odors produced from mechanical damage, likely caused during the collection of branches, rather than to chemicals released in response to *A. tsugae* feeding. Furthermore, they found that *L. nigrinus* collected from stands of western hemlock (comparable to my Cohort 1: PNW) responded to odors from both western and eastern hemlock but had a strong preference for western hemlock. However, beetles that had been reared in the laboratory on *T. canadensis* for three generations at the Biological Rearing Facility at Virginia Polytechnic Institute and State University (and sent overnight to the test sight at Oregon State University Integrated Forest Protection Laboratory) exhibited a general lethargy and unresponsiveness to odors from either tree species.

In another olfactometer study, Arsenault (2013) contradicted some of Wallin et al. (2011) findings in that *L. nigrinus, L. nigrinus* x *L. rubidus,* and *L. osakensis* all responded to both *A. tsugae* and eastern hemlock foliage, with the strongest response being to infested foliage. Arsenault (2013) suggested that differences in results might be due to adaptation of introduced *L. nigrinus* to *A. tsugae* on eastern and Carolina hemlocks in the Southern Appalachians. The *L. nigrinus* used by Arsenault (2013) were wild-caught near Asheville, NC and Banner Elk, NC, which are comparable to Cohort 2 (NC) in my study. I suggest that the differences between these two studies may partly be the result of the

origin of the beetles used. Further research examining differences in *L. nigrinus* response to eastern versus western hemlock species when infested by *A. tsugae* may be an important component in our understanding of the success and survival of laboratory-reared *L. nigrinus* released into Eastern U.S. hemlock forests.

CHAPTER THREE

METHODS AND MATERIALS

Laricobius nigrinus adults were reared for eight weeks each in the spring of 2013 and 2014 to determine and compare fecundity of four cohorts, two wild-caught and two F_1 laboratory-reared generations from each wild-caught cohort. Cohorts examined included:

- Cohort 1: Wild-caught *L. nigrinus* from Seattle, WA (Pacific Northwest) (PNW cohort) – native population
- Cohort 2: Wild-caught *L. nigrinus* from Banner Elk, NC (NC cohort) – naturalized population
- Cohort 3: Laboratory-reared *L. nigrinus* F_1 generation from Seattle, WA parents (PNW-F_1 cohort)
- Cohort 4: Laboratory-reared *L. nigrinus* F_1 generation from Banner Elk, NC parents (NC-F_1 cohort)

Individual *L. nigrinus* cohorts were maintained separately throughout the experiment.

Laricobius nigrinus Adult Collection:

R. McDonald of Symbiont Biological Pest Management (Sugar Grove, NC) was hired to collect wild-caught cohorts from the Pacific Northwest (PNW) in both 2013 and 2014. In 2013, two days of collection yielded 538 *L. nigrinus* adults. Beetles collected the first day were stored overnight in a hotel refrigerator and shipped overnight following the second day of collection. The beetles were shipped in a cardboard chowder container on excelsior with a cooling pack inside of the insulated container to Seneca, SC and driven

to the Clemson University Insectary (L. Burgess, personal communication, August 13, 2014). In 2014, *L. nigrinus* adults were shipped overnight from Seattle, WA on February 12, 2014 and arrived February 13, 2013 to The University of Georgia, Athens, GA. Between 200 to 300 adult *L. nigrinus* were shipped inside fishing bait containers, purchased in Seattle, WA, which contained pieces of *A. tsugae* infested hemlock for food. In Athens, beetles were misted and stored in the containers in which they had arrived. Containers were kept in an incubator set to 6^{o}C day temperature, 4^{o}C night temperature, 12:12 light:dark, and 65% RH. On February 24, 2014, *L. nigrinus* were processed into release containers (rectangular plastic containers with screened lids for ventilation) with 50 beetles in a container and approximately four 20 cm long *A. tsugae* infested hemlock twigs for food (A. Mercer, personal communication, August 14, 2014, Y. Zhang, personal communication, August 13, 2014). These beetles were picked up and driven to the Clemson University Insectary on February 27, 2014 by A. Ferguson.

M. Wells (2013), J. Burgess (2014), and I (2013 & 2014) (Clemson University Insectary) collected wild-caught cohorts in North Carolina from lower branches on hemlock trees and hedges in urbanized areas of Banner Elk, NC. Beetles were collected using either an inverted umbrella (2013) or white beating sheet (2014), which was held under hemlock branches by one person while another person beat the branches forcefully with a stick. Beetles were collected into a vial connected to an aspirator. Containers for holding the beetles and *A. tsugae*-infested hemlock twigs, with holes for ventilation, were brought from the Clemson University Insectary to North Carolina. Once beetles were collected and counted, 356 beetles in 2013 and 101 beetles in 2014 were placed in the transport

containers, which were stocked with *A. tsugae*-infested hemlock twigs. Beetles were kept inside these containers until they were returned to Clemson University. In 2013, beetles were held overnight in a refrigerator before returning them to Clemson University. In 2014, beetles were brought to Clemson the day of collection. In both 2013 and 2014, containers of beetles were placed in a cooler and surrounded by cold packs while in transit.

For both years of the experiment, once field-collected (PNW and NC) beetles were brought to the Insectary, they were placed in temporary containers, with *A. tsugae*-infested twigs. Beetles were kept in a climate-controlled space at 4^{o}C; 10:14 (light:dark); 60% RH during the pre-oviposition period. In 2013, PNW beetles were set up in reserve containers in the Clemson University Insectary on February 12, 2013, four weeks before the first week of the study (March 13, 2013). NC beetles were brought in to the Insectary on March 1, 2013, approximately two weeks before the first week of the study. In 2014, PNW beetles were brought to the Clemson University Insectary on February 27, 2014, almost two weeks before the first week of the study (March 10, 2014). NC beetles were brought in to the Insectary on February 22, 2014, approximately 2.5 weeks before the first week of the study.

Storage of *L. nigrinus* 2013:

Beetles used in both laboratory-reared F_1 cohorts in the 2013 study were the filial generation of wild-caught beetles reared at the Clemson University Insectary in 2012. Adults to be used for the two laboratory-reared cohorts in spring of 2013 were stored from the time of their emergence from the soil (times varied) until the first week of the experiment in one-gallon (3.7854L) glass jars (Fungi Perfecti, LLC, ECAJ1G/4, Olympia, WA) with 25 adults per jar. The plastic lid of each jar had an approximately 6.5 cm diam. circle cut out and Polyester Noseeum Netting (625 holes / 2.54 cm^2, Army Navy Store, IN-008, Barre, VT) hot-glued to the inside circumference to allow air exchange (Fig. 2 A & B). Two 9 cm diam. pieces of white, smooth, Grade No. 613 filter paper (VWR, Radnor, PA) were placed in the bottom of each jar to absorb condensation. *Adelges tsugae*-infested hemlock bouquets used in the jars were constructed as follows. Cylinders (approx. 6.3 cm high x 3.5 cm diam.) of Artesia floral foam (FloraCraft®, Ludington, MI) were placed in a tub of tap water for four to six weeks until they were fully saturated. Saturated cylinders of floral foam were placed inside plastic cylinders (6.3cm high x 4.0 cm diam.), which were then filled to the brim with tap water. The top of the plastic cylinder was covered by a double layer of clear plastic ClingWrap (GLAD, Oakland, CA) secured to the cylinder by a #64 rubber band (Wal-Mart Stores, Inc. Bentonville, AR) to prevent adult beetles from burrowing into the floral foam. Any ClingWrap extending beyond the rubber band was removed to help prevent *L. nigrinus* adults from becoming trapped (Fig. 3 A). Fifteen to twenty hemlock twigs, approximately 16.5 cm long, were inserted through the ClingWrap into the foam cylinder (Fig. 3 B &

C). *Adelges tsugae* infested hemlock twigs used in this study were field-collected in South Carolina and Georgia.

In addition to *A. tsugae*-infested twigs, four to five 0.5 cm x 1.5 cm strips of food supplement were added atop the foliage. Food supplement was a mixture developed by Cohen & Cheah (2011) to mimic the nutritional requirements of *L. ngirinus* provide by *A. tsugae*. This mixture is made exclusively for *A. tsugae* predator-rearing laboratories and was freeze-dried before shipping from North Carolina State University. The food supplement was stored in a refrigerator at the Clemson University Insectary. The food supplement was prepared by mixing it with honey to form a paste and spreading it between two strips of Parafilm (Parafilm, Bemis Flexible Packaging, Neenah, WI). The food supplement was then stored in a freezer and food strips were cut up and added to foliage when needed (Fig. 4).

These jars were unassembled once every two weeks by removing the bouquet from individual jars, and each twig was carefully searched for adult beetles. This was done under a mosquito net to prevent *L. nigrinus* from escaping (Fig. 5). *Laricobius nigrinus* were handled using 10/0 camel hair paintbrushes (Daler <> Rowney Limited, Bracknell, Berkshire, U.K.) or aspirators and were temporarily placed into a holding container 3.8 cm high x 5.5 cm diam. with a ventilated lid. The ventilation hole was 2.5 cm diam. and covered with Polyester Noseeum Netting hot-glued to the inside of the lid (Fig. 6). Once all beetles had been recovered, or accounted for if any had died or were missing, the beetles were transferred to a newly set up jar.

By mid-February 2014, 84 emerged filial adults from Cohort 1 (PNW) and 60 emerged filial adults from Cohort 2 (NC) from the previous year had been kept and maintained in the insectary to be used in Cohort 3 (PNW-F_1) and Cohort 4 (NC-F_1), respectively, for the study in 2014. Some emerged adults from the 2013 PNW cohort were used for normal insectary rearing outside the scope of this project, and the remaining 2013 PNW and NC filial adults were released in infested hemlock forests. The 2013 F_2 adults from Cohorts 3 (PNW-F_1) and 4 (NC-F_1) were released into the field after numbers were recorded or were used to practice determining gender of live beetles. Beetles used to practice determining gender were frozen and sexed to double check gender. Some of the F_1 emerged adults from the 2013 NC cohort (Cohort 2) were also used to practice determining gender of live beetles.

Figure 2: (A) Open glass jars with filter paper and lids used as reserve containers for adult *L. nigrinus* and as oviposition jars. Ventilated lids are shown on the tray adjacent to the jars. (B) Closed reserve and oviposition jar showing filter paper and hemlock bouquet (2013).

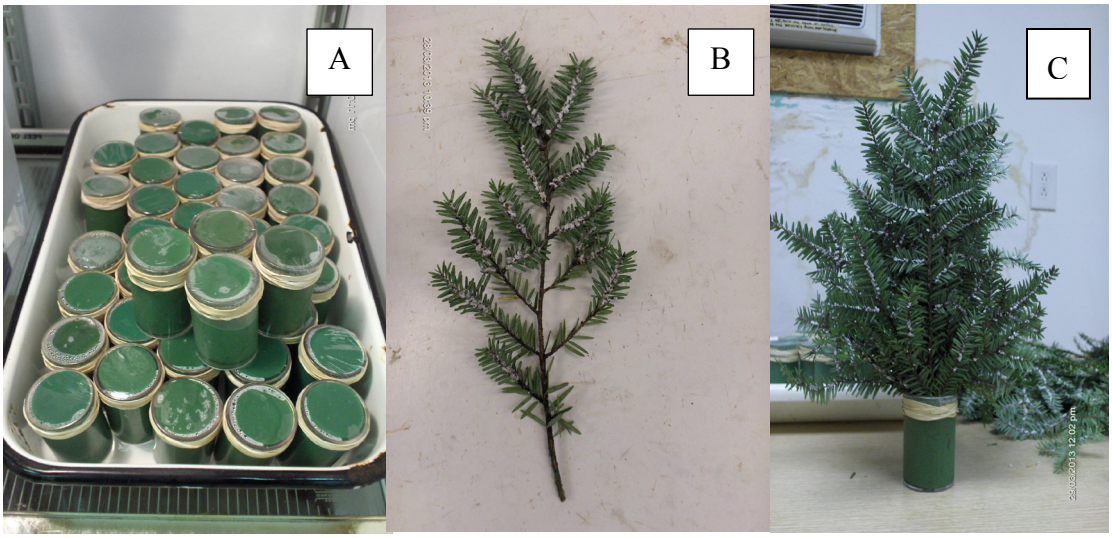

Figure 3: (A) Saturated floral foam cylinders inside plastic containers for use in reserve and oviposition jars. (B) *Adelges tsugae* infested hemlock twig used to make bouquets used in the reserve and oviposition jars. (C) Saturated floral foam cylinder with bouquet of *A. tsugae* infested hemlock twigs.

Figure 4: *Laricobius nigrinus* food supplement cut into strips.

Figure 5: Workstation showing mosquito-netting cover used to prevent adults from escaping while being handled.

Figure 6: Temporary holding container for beetles and paintbrush for handling *L. nigrinus* adults.

Storage of *L. nigrinus* 2014:

In 2014, a severe shortage of available *A. tsugae*-infested hemlock in nearby collection sites forced the Insectary to economize on available food in all steps of the rearing process. During storage, in place of the one-gallon holding jars used in 2013, adult *L. nigrinus* were held in smaller containers, which contained enough food for the beetles to survive, but less food than would be supported by the one-gallon glass jars. This model helped to prevent a surplus of food for beetles, which was critical for successful rearing in 2014. Adult beetles were initially held in 2 oz (59.147 mL) clear plastic BPA free containers (diamond TM copyright 2012 Hearthmark, LLC dba Jarden Home Brands, Daleville, IN 47334). Lids were hole-punched using a round 6.3 mm diam. Recollections™ Hand Punch (Michaels Stores, Inc., M10282473, Irving, TX), at least four times, creating an asymmetrical quatrefoil-shaped hole, between 1 cm and 2 cm across. Polyester Noseeum Netting was hot-glued over the holes on the inside of the lid to allow for air circulation and to prevent escape by adults. Each 2 oz (59.14mL) container held between one and 25 adults for a period of no longer than 11 days. The 2 oz (59.14 mL) containers served as temporary holding containers until more suitable long-term holding containers could be found. In the first few weeks up to 25 adults were kept in each 2 oz (59.14 mL) container. However, once suitable containers were created, adults were placed in the 2 oz (59.14mL) containers until there were enough beetles available (15 beetles) to fill the larger, long-term containers. Longer-term containers into which adults were transferred were 4 oz (118.29 mL) clear flexible polypropylene, BPA free containers (Newspring 19519504 Ellipso Clear 4 oz (118.29 mL) container with lid –

500/CS, Lake Forest, IL) with a 2.5 cm diam. hole drilled through the lid and Polyester Noseeum Netting hot glued inside to cover the hole. Each 4 oz (118.29 mL) container held up to 15 beetles (Fig. 7). Both 2 oz (59.14 mL) and 4 oz (118.29 mL) containers were filled to the brim with *A. tsugae* infested twigs up to 5 cm long and up to 7.5 cm long respectively, with one to three strips of food supplement placed atop the foliage. As twigs were not inserted into saturated floral form, they dehydrated in approximately 14 to 18 days, at which time more twig pieces were cut up and placed atop the dried twigs. Since containers were not disassembled at this time, dried twigs were not removed. Approximately once monthly, the 4 oz (118.29 mL) containers were disassembled and all beetles were collected. This was done under the mosquito netting covers as described above.

Figure 7: Top view of 2oz (59.14 mL) (left) and 4 oz (118.29 mL) (right) containers for holding adults prior to oviposition (Fall 2013 – Spring 2014).

Gender Determination of Adults:

Prior to 2013, the literature indicated that the gender of *L. nigrinus* adults could not be determined without dissection because the genitalia are typically retracted into the body (Salom et al., 2012, Zilahi-Balogh, 2001). Because of that, in 2013 adult gender was not

determined prior to establishment of oviposition cohorts. To determine gender of adults used in 2013, dead adults recovered during each week of the experiment were preserved in individual vials containing 100% EtOH. Vials were labeled with week and individual oviposition jar number. Because many beetles had become hard and brittle during EtOH storage they were placed in a 10% KOH solution overnight prior to dissection. Although this greatly facilitated determination of gender, placing them in KOH prevented any future genetic analyses of these individuals. All surviving adults at the conclusion of the experiment were frozen then dissected to determine gender. Gender was determined by dissection at 50X magnification (Motic, Speed Fair Corporation, Ltd., Hong Kong; Light source: CHIU Technical Corporation LUMINA, Kings Park, NY) (Fig. 8). Adult *L. nigrinus* were positioned with the ventral side facing up and held in place by pressing down on the elytra with fine tipped forceps (BioQuip Products, 4524, Rancho Dominquez, CA) (Fig. 9 A). The abdomen of each adult was dissected using insect pins.

In the fall of 2013, the Clemson University Insectary staff attended training sessions held at the University of Georgia to learn techniques (Shepherd et al., 2014) that allow gender determination of live *L. nigrinus.* My techniques were modified by holding *L. nigrinus* in place using sticky flags (Post-it, Arrow Flags, 684-ARR1, Distributed by Wal-Mart Stores, Inc. Bentonville, AR) with the abdomen facing up, rather than cooling the beetles to slow them down as suggested by Shepherd et al. (2014). Gender determination of live beetles was practiced on over 170 adults prior to the 2014 rearing season. All beetles used

in the 2014 experiment had gender determined using this method so that I could establish oviposition pairings having 1:1 male:female ratios.

In 2014, when a dead beetle was found in the colony, gender was determined prior to placing it into 100% EtOH. At the conclusion of the eight-week study, I determined the gender of ten live adults (five male and five female) from each cohort, which were then frozen and stored. After determining the gender of all remaining adults they were released into the field.

Dead beetles collected during both 2013 and 2014 were preserved in EtOH to be deposited in the Clemson University Arthropod Collection (CUAC) as voucher specimens for this study.

Figure 8: Microscope used for counting eggs and determining gender of *L. nigrinus.*

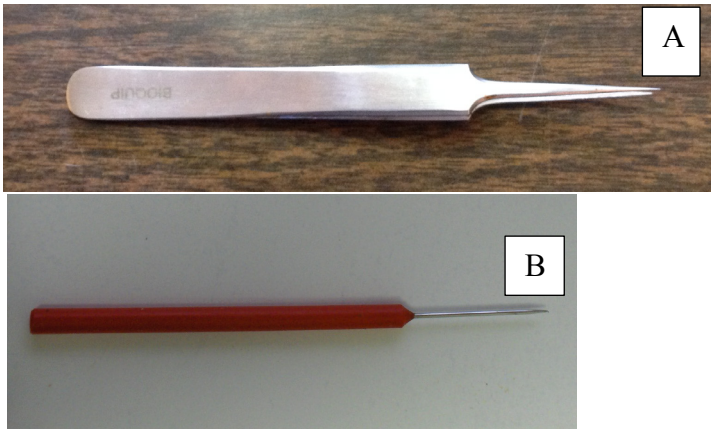

Figure 9: (A) Fine tipped forceps used in counting eggs in 2014 and to hold dead beetles for sexing. (B) Dissecting needle used in counting eggs (2013).

Rearing Conditions:

I. Oviposition Jars

In each trial, eight one-gallon (3.7854L) glass jars were used to maintain adult *L. nigrinus* during oviposition. Each oviposition jar was given a unique number that distinguished it from other oviposition jars; the jar number was kept the same for the same group of beetles throughout the experiment, i.e. beetles in jar number "1" would be moved into a newly-assembled jar numbered "1" each week.

In 2013, each oviposition jar bouquet consisted of ten to eleven *T. canadensis* twigs, approximately 16.5 cm in length that were heavily infested with *A. tsugae*. Because the number of beetles in 2014 was reduced from 30 beetles per oviposition jar (2013), to four beetles per oviposition jar, each oviposition bouquet held one to two such twigs (two twigs per bouquet when four *L. nigrinus* adults were alive, one twig if adult numbers declined to two beetles). This reduction in the number of beetles in the study, and

therefore the amount of food required during oviposition, ensured that the limited food that year did not affect rearing.

Each cohort was set up and dismantled once every seven days on a different day of the week, Monday through Thursday (Fig. 5). A given cohort was always dismantled on the same day of the week. Each jar was dismantled by removing the bouquet under a mosquito net. Each oviposition twig was carefully searched for adult beetles while taking care not to dislodge either *A. tsugae* ovisacs or *L. nigrinus* eggs. *Laricobius nigrinus* were handled using 10/0 camel hair paintbrushes, and were temporarily placed into a plastic container as was done when reserve jars were dismantled (Fig. 6). Once all live beetles had been recovered, or recorded as either dead or missing, the beetles were transferred to a newly set up oviposition jar with the same jar number for the next week.

Oviposition jars were held in Percival (model I-30 BLL) environmental chambers (Percival, Boone, IA) at 8^{o}C; 12:12 (light:dark); 60% RH. Percival chambers were maintained at the same conditions both years. In 2013, each Percival contained two shelves and held up to 18 jars. In 2014, the Clemson Insectary began using larger Percival (model 136 VL) chambers, which had four shelves each of which held all eight jars for a cohort (Figs. 10 A & B).

Each week, after jars were dismantled, the foliage from the old oviposition jars was searched for *L. nigrinus* eggs using a microscope at 12X magnification. *Adelges tsugae*

ovisacs were teased open using a dissecting needle in 2013 (14 cm long by 0.5 mm diam.) to search for *L. nigrinus* eggs (Fig. 9 B). *Laricobius nigrinus* eggs can be distinguished from *A. tsugae* eggs by the former's yellow coloration and larger size (Fig. 11). In an attempt to improve accuracy when counting eggs, in 2014 fine tipped forceps (Figure 9 A) were used.

Figure 10: (A) Percival (door open) showing nine jars from a cohort (2013). (B) Inside of Percival growth chamber with empty oviposition jars (2014).

Figure 11: *Laricobius nigrinus* egg (yellow) in *A. tsugae* ovisac with brown-red *A. tsugae* eggs (Lamb, n.d.).

II. Larval Rearing

2013

In 2013, hemlock twigs having up to 375 *L. nigrinus* eggs were selected weekly from each cohort. These twigs were inserted into two fully saturated floral foam blocks (7.5 cm × 11 cm × 23 cm) covered by ClingWrap held in place with Scotch Tape (Scotch 81011296 Magic Invisible Tape, Hutchinson, MN). Blocks were placed in rearing tents (BugDorm-2120F Insect Rearing Tent, fine mesh, 60 cm x 60 cm x 60 cm, MegaView Science Co., Ltd., Taichung, Taiwan) atop wire mesh cut to fit the base of the tent in order to lend additional support for the blocks (Figs. 12 & 14). The top floral foam block contained several rows of twigs from the oviposition containers inserted on either side. These twigs were in a horizontal orientation with the dorsal side of the twig oriented upward as it would be in nature. Between each row of twigs having *L. nigrinus* eggs were one to two rows of field-collected twigs infested with *A. tsugae* as a food source for newly hatched *L. nigrinus* larvae. Therefore, twigs with eggs were inserted in multiple rows on either side of the block, with space between each row for feeding twigs. Feeding twigs were oriented horizontally, rotated 180^{o} to their orientation in nature with the twig ventral side up, so that they touched the twigs with eggs that were above and below them. This allowed *L. nigrinus* larvae to move on to the additional twigs for feeding. Once all of the oviposition twigs were inserted into the blocks, there was generally space on the lower edges of the blocks. This space was filled with additional feeding twigs. The bottom block contained only feeding twigs oriented horizontally but rotated 180^{o} to their orientation in nature. Each block contained approximately 135 total twigs. The twigs in

the lower block provided additional *A. tsugae* eggs for developing *L. nigrinus* larvae (Figs. 12 & 13). *Adelges tsugae* infested twigs for oviposition and feeding had been collected in the field five days to four weeks prior to their use in the oviposition jars and the larval tents. Feeding twigs were kept in saturated floral foam inside of a clear plastic tub filled about halfway up the floral foam with water. Twigs were kept in the same environmental conditions as larvae.

Oviposition twigs containing up to 375 *L. nigrinus* eggs were placed together in a larval rearing tent. Twigs were selected such that each oviposition jar from a given cohort contributed at least a few twigs to the total number placed inside tents established that week. Tents were placed on shelving with custom-made wooden frames that supported the outer base of the tent while providing access to a larval collection jar suspended from the bottom of the tent. Each tent had a 1-pint (473.18 mL) glass jar (Mason Golden Harvest Self Sealing Jar, Kerr Glass Manufacturing, Portland, OR) that was painted black attached to the flexible tent bottom. A hole the size of the circumference of the collection jar mouth was cut out near the center of the base of each tent. The inside of the lid of each jar was removed. The hole at the base of each tent was covered with wire mesh hot-glued over the hole on the inside of the tent (this allowed larvae to fall into the jar while preventing twigs from falling in). The circumference of the jar lid was glued to the circumference of the hole outside of the tent. This arrangement allowed the jar to be screwed on and off of the tent bottom. The weight of the jar created a funnel shape in the tent floor that caused mature larvae to move to the mouth of the jar after they dropped

from the foliage prior to pupation (Fig. 14). The black color of the jar simulated the soil where larvae naturally move prior to pupation in the field (Lamb et al., 2005). Each tent was labeled with the cohort, number of eggs, and date it was set up.

If fewer than 375 *L. nigrinus* eggs were collected from a given cohort in any week, all oviposition twigs were placed together inside a tent. All eggs produced by adults in the reserve jars, and any eggs above the 375 used for rearing were released in *A. tsugae* infested forests in SC and GA.

In 2013, a total of thirty-two larval tents were set up. Tents were kept in an environmentally controlled room at 13^{o}C, 12:12 (light:dark), 60% RH. After a larval tent was set up, it was checked daily until no larvae had dropped for a period of at least four days. Although tents were checked daily, it was expected that the majority of larvae would drop between days 25 and 50, which is when according to the Clemson University Insectary 2005 rearing data, on average 94.6% of mature live larvae could be expected to drop from the foliage (based on data from Seattle, WA beetles). Larvae dropping from twigs were recorded as mature, immature, or dead. Mature larvae were determined by their yellow coloration on the ventral side, sclerotized dorsal side, relative lack of white wool on the dorsal side, and negative phototaxis. I also observed that mature larvae generally curl into a ball when disturbed with a camelhair paintbrush. Immature larvae were determined by a lack of yellow ventral coloration and greater retention of white

wool on the dorsal side. I observed that these larvae would generally arch their body when touched with the paintbrush and did not curl into a ball. In 2013, dead larvae were placed in vials containing 100% EtOH labeled by tent number and cohort, and immature larvae that had left the foliage were placed into pupal-aestivation containers along with mature larvae. Larvae were placed into pupal-aestivation containers (see below) separated by cohort, tent, and date.

Figure 12: Side view of larval rearing tent (2013).

Figure 13: Close up of twigs inside larval rearing tent (2013).

Figure 14: Rearing tents containing developing larvae and feeding twigs. Mason jars, painted black, for collection of mature larvae are attached to the bottom of the tent (2013).

2014

In 2014, because of the lower number of *L. nigrinus* adults per oviposition jar, each jar in any given cohort had a correspondingly numbered larval box in which any eggs produced were placed. Twigs were placed into a new corresponding larval box every week. This allowed me to determine the number of larvae produced per jar during each week of the study. In 2014, each oviposition jar within a cohort represented one of the eight replicates for that cohort. Because of the large quantity of larval containers needed (4 cohorts * 8 oviposition jars per cohort * 8 weeks = 256 larval containers) custom designed larval rearing boxes (Fig. 15) replaced the larger larval rearing tents used in 2013. Boxes were designed by D. Cottrell) and assembled by K. Byrd at the Sonoco Institute, Harris A.

Smith Building, Clemson University. Oversight of box design and assembly was provided by Dr. R. A. Hurley, Department of Food, Nutrition and Packaging Sciences, Clemson University. Each box measured 17 cm W x 23 cm H x 32 cm L and was made of C-Flute corrugated cardboard (Pratt Industries, Greenville, SC). The cardboard was digitally diecut from flat sheets using a Kongsberg XN sample table. The boxes had openings measuring 26.0 cm x 13.0 cm on each side and an 11.4 cm x 12.5 cm opening on the end. All openings were covered by Polyester Noseeum Netting to allow for air exchange and light penetration inside the box. They had a removable lid made of E-Flute corrugated cardboard (Pratt Industries, Greenville, SC) with a screened ventilation opening of 27.2 cm x 12.0 cm. The boxes also had a 7.6 cm x 7.6 cm x 10.8 cm opening in one end which was used to hold one floral foam block held 10.7 cm above the base of the box opposite the end having the screened opening.

A saturated floral foam block (approximately 7.6 cm x 7.6 cm x 10.7 cm) was wrapped in plastic wrap as described above for 2013 (Fig. 16). Hemlock twigs with approximately 200 *A. tsugae* ovisacs were inserted toward the bottom of the floral foam block so that they were below, but touching *L. nigrinus* oviposition hemlock twigs from the oviposition jars in the same block. Twigs having *A. tsugae* ovisacs were placed horizontally rotated 180^{o} to their orientation in nature, toward the bottom of the floral foam, while oviposition twigs were placed slightly above the feeding twigs while still touching them, in a horizontal orientation, but with the top of the twig oriented upward as it would be in nature. Twigs used for either feeding or oviposition were stored in the

same environmental room as the larval boxes and kept in saturated floral foam until they were needed.

Larval boxes had a removable heavy-duty brown Kraft paper (Pratt Industries, Greenville, SC) tray in the bottom used for collecting mature, immature, and dead larvae as they dropped off the twigs. Because the boxes did not sealed perfectly, in order to reduce the possibility of larvae escaping, I placed Scotch tape over all obvious openings. In addition, there was a flap made from a portion of the box front covering the access opening for the removable tray which was held closed either by Velcro (Velcro Industrial Strength Sticky-Back Hook and Loop Fastener Strips, VEK90199, Velcro USA, Inc. Manchester, NH) or rubber bands (17.78 cm x 0.31cm Advantage File Bands, Alliance Rubber Company, #117B, Hot Springs, AR) (Fig. 15 B). Unlike the larval tents in 2013, larval boxes in 2014 did not need to be checked daily due to the lower number of eggs in each box. Larval box trays were checked every five days from the day oviposition jars were broken down until approximately 25 days later, at which time larvae were expected to begin dropping from the foliage. If any larvae dropped before the 25th day, the box was checked again the following day. Trays were checked daily between days 25 and 50. Boxes were placed on wire shelving units having seven shelves per unit. Seven larval rearing boxes fit on each shelf. Because boxes placed on middle and lower shelves were shaded by boxes on upper shelves, I assigned boxes within each cohort to spaces across all shelves so that no single cohort had all larval rearing boxes on either the top, middle, or lower shelves.

Larval boxes were placed in an environmentally controlled room maintained at 13^{o}C, 12:12 (light:dark), and 60% RH (Fig. 17). In 2014, a total 256 larval rearing boxes were set up. All larvae dropping from twigs were recorded as mature, immature, or dead. Mature and immature larvae were determined using the criteria as in 2013. In 2014, dead larvae were not stored in EtOH but were held collectively by cohort in petri dishes. Dead larvae are poor specimens for genetic analysis as the day of their death before dropping was unknown, and DNA could have begun to deteriorate by the time they were found. Larvae had been preserved in EtOH in 2013 in case I determined that I needed to conduct genetic analyses on them. For 2014, I determined that larvae would not be genetically analyzed during my study, eliminating the need to store dead larvae in EtOH. As dead larvae could be preserved dry, larvae not stored in EtOH were kept in petri dishes for records. Four larval specimens were placed in EtOH vials for long-term preservation, before it was decided that storing specimens dry was preferable.

As in 2013, immature larvae were placed into pupal-aestivation containers along with mature larvae. Larvae were placed into pupal-aestivation containers (see below) separated by cohort and date.

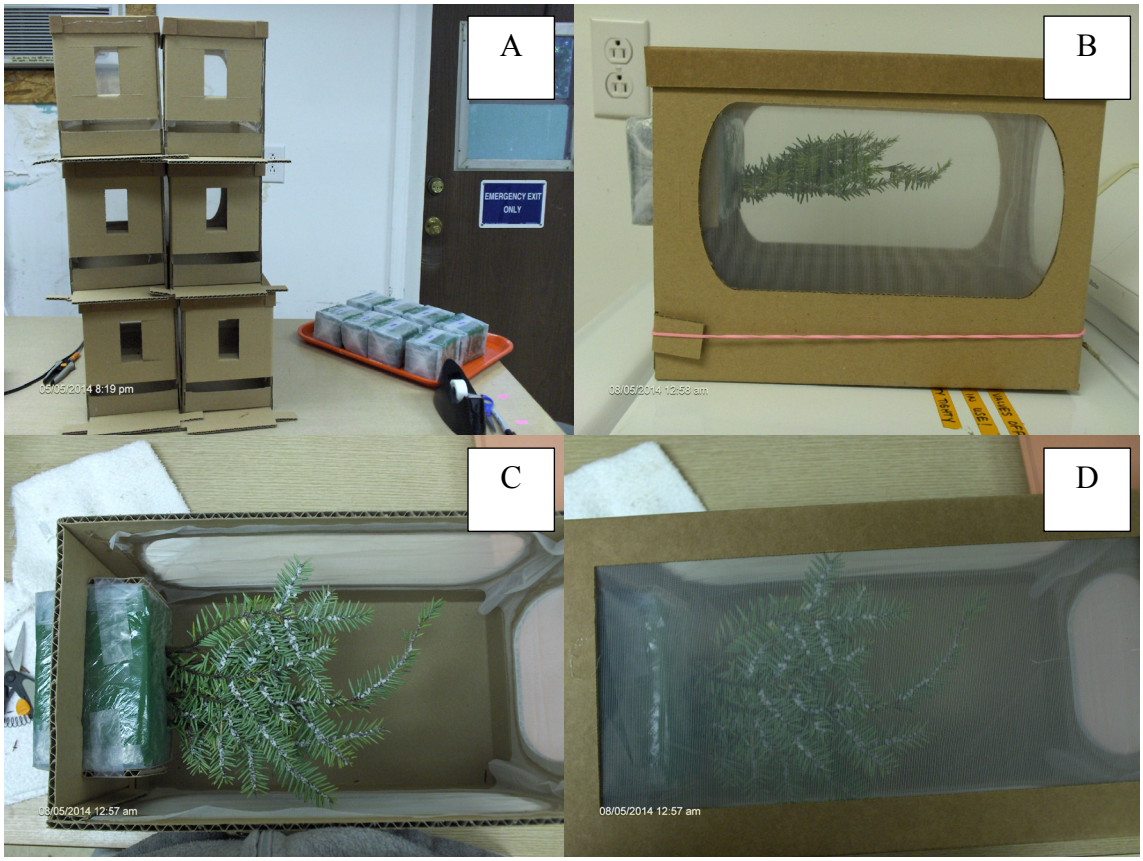

Figure 15: (A) Empty larval rearing boxes. Rectangular hole was for insertion of floral foam block. (B) Side view of larval box showing the flap over the removable tray held closed by a rubber band. Floral foam and feeding twigs can be seen inside. (C) Top view of larval rearing box with top removed showing floral foam block and feeding twigs. (D) Top view of larval box with top in place showing floral foam block and feeding twigs.

Figure 16: Floral foam blocks wrapped with Cling Wrap and Scotch Tape used for holding oviposition and feeding twigs in larval rearing boxes (2014).

Figure 17: Larval rearing boxes placed on shelving units in the environmentally controlled rearing room (2014).

III. Pupal-Aestivation Containers

Mature larvae began dropping from the foliage during April in both 2013 and 2014, and were placed in clear plastic containers (Newell Rubbermaid Inc. TakeAlongs, Atlanta, GA) (base: 11.43 cm × 11.43 cm; height: 8.89 cm; top: 15.24 cm × 15.24 cm) for pupation. To provide ventilation in the containers, two 8 cm × 4 cm rectangles were cut out on opposite sides of each container and a 13 cm × 4 cm rectangle was cut out of the lid. Openings were covered by Polyester Noseeum Netting (625 holes / 2.54 cm^2) hot-

glued on the inside of the container. Each container held approximately 5 cm of a soil mixture (2000 mL sphagnum peat moss, 500 mL natural play sand (Pavestone, Tyrone, Georgia), 1000 mL distilled water). The first cm of soil mixture was spread evenly along the bottom and lightly compressed, and approximately 3.5 cm of loose soil mixture was added on top (Fig. 18).

As larvae were collected from each larval rearing tent (2013) or larval rearing box (separated by cohort) (2014) they were placed cumulatively on the soil mix until a total of up to 50 larvae from a single cohort were in each container (Fig. 19). In 2013, the first container was established on April 9th and the final container on July 4th. In 2014, the first container was set up on April 6th and the final container on June 19th. Dates were dependent on when larvae began dropping and ceased dropping from the larval rearing chambers. Larvae in soil were held in a climate-controlled room at 15^{o}C; 12:12 (light:dark). If a container did not have 50 larvae by 10 days following placement of the first larva in the container no more larvae were placed into the container. This allowed the containers to hold larvae that may be expected to emerge as adults in the fall within weeks of each other, while economizing on containers. All pupation containers were lightly misted with distilled water once every six days to maintain soil moisture. After 41 days at 15^{o}C; 12:12 (light:dark) each pupal container was transferred to another environmental chamber maintained at 19^{o}C; 16:8 (light:dark) and misted twice weekly (on Tuesdays and Fridays in 2013 and on Mondays and Thursdays in 2014). Once they drop from the foliage, mature *L. nigrinus* larvae pupate within 36 hours (Salom et al.

2012), then eclose as adults in approximately two weeks but remain in the soil until early autumn (September to February).

Figure 18: Pupal-aestivation containers containing soil mixture.

Figure 19: Larvae on white paper during collection (2013).

IV. Emerging Adults

Emergence of *L. nigrinus* adults in the field is triggered both by changes in temperature and photoperiod (Lamb et al., 2007). This change is mimicked in the Insectary to coincide with naturally occurring environmental changes cueing beetle emergence in the field. In insectary rearing, the goal is to match natural conditions so that adults begin to emerge around the time *A. tsugae* begin to come out of aestivation in the field. To

accomplish this, aestivation containers were transferred to another environmentally controlled room and maintained at 13^{o}C; 8:16 (light:dark), and 60% RH to trigger adult emergence approximately 124 days after the transfer to the 19^{o}C; 16:8 (light:dark) conditions **(**Fig. 20). Following our Insectary's protocol, *L. nigrinus* adults typically emerged from the soil in the aestivation containers between early October and late November. However, because some pupal containers established toward the end of larval maturation continued to exhibit adult emergence past the expected date of final adult emergence, I continued weekly misting of those containers until early February 2014. Adult emergence from the soils mix was recorded daily.

Figure 20: Pupal-aestivation containers with emerging adults in a climate controlled space (2013).

Experimental Design 2013:

Data were collected on each cohort from eight oviposition jars, each jar containing 30 adult *L. nigrinus* for a period of eight weeks. Gender of beetles was not determined prior to the study in 2013, and the beetles were kept at 30 per jar in order to ensure adequate numbers of males and females were present in each jar for rearing purposes. If there were more than 240 beetles available for a cohort, those not used in the eight oviposition jars were placed in reserve jars and maintained under the same conditions as those in the oviposition study. Reserve beetles were used to replace dead or missing beetles to maintain the original cohort size of 30 adults per oviposition jar throughout the study. If the total number of beetles in a given cohort in both oviposition and reserve jars fell below 240, the highest numbered oviposition jar (e.g. Jar 8) became a reserve jar and those beetles were used to replace dead or missing beetles in the remaining oviposition jars (e.g. Jars 1-7). Because sexing methods for live larvae were unavailable prior to the study 2013, the replacement of beetles was deemed necessary although it was not ideal for the purposes of an experimental study. Every seven days, all jars from each cohort were disassembled and adult *L. nigrinus* counted. Live adults were placed into new jars with a fresh bouquet of *A. tsugae* infested hemlock twigs, and any additional beetles needed to maintain 30 individuals per jar were added.

L. nigrinus eggs from the previous week were counted under the microscope at 12X magnification. I found that my egg counts underrepresented the actual numbers of eggs once larvae began to mature and drop from the twigs prior to pupation. I attribute this

discrepancy to human error due to the large number of twigs (approximately 320 twigs per week) that needed to be carefully searched for *L. ngirinus* eggs coupled with the need to set these twigs up in larval containers before eggs began to hatch. Because of the difference between egg counts and the number of mature larvae collected, I did not use 2013 egg data in any analyses.

The 10 to 11 twigs from the hemlock bouquet from each oviposition jar were placed collectively into a single larval rearing tent and held at (13^{o}C, 12:12 (light:dark), 60% RH) (Figs. 12 & 14). Twigs from oviposition jars that had more than 375 eggs, and twigs from reserve jars were released directly into the field after one week of adult oviposition. No data was collected from the reserve jars.

Experimental Design 2014:

Gender of adult *L. nigrinus* was determined during the week before the study was initiated. Single female/male pairs were placed individually into 89 mm petri dishes and provided with two to three *A. tsugae*-infested twigs, approximately 80-85 cm long. Petri dish lids had a 1 cm diam. hole drilled near the center which was covered with Polyester Noseeum Netting glued to the inside of the lid to allow for air exchange. The circumference of the closed petri dish was sealed with Parafilm. During the first week of the study, two female/male pairs were moved from the petri dishes and placed inside a single oviposition jar. Each cohort consisted of eight oviposition jars (Fig. 3). Therefore, each of the eight jars per cohort contained two females and two males, and one 16.5 cm

A. tsugae-infested twig for every two beetles. (In the first week, some of the twigs, which were slightly longer than 16.5 cm were cut into smaller twigs to prevent *A. tsugae* ovisacs from being compressed by the jar lid; each piece was labeled as a separate twig, but the overall *A. tsugae* density was equivalent to that of approximately two twigs in any other week.) When a replicate lost more than one beetle (reducing the total per jar to <3) at the next jar change only one twig would be placed in the oviposition jar. In 2014, dead or missing beetles were not replaced. Every seventh day, eight jars from each cohort were disassembled using the methods described above. This was done on Monday through Thursday, with one cohort handled per day. All live adults were placed into new oviposition jars with a fresh bouquet of *A. tsugae*-infested hemlock twigs. Twigs from the previous week were searched for *L. nigrinus* eggs using fine tipped forceps under a microscope at 12X magnification and numbers were recorded. Twigs from individual jars were placed into floral foam blocks containing additional *A. tsugae* infested twigs and maintained in the custom designed larval boxes. Boxes were labeled as W1.3A with W indicating "week", 1 indicating that it was from a jar in the first week, 3 indicating Jar 3, and A indicating the box belonged to the NC-F_1 cohort (B=PNW, C=PNW=F_1, D=NC) (Figs. 15 & 17). This was done for eight weeks.

Data Collection:

The primary goal of my study was to examine *L. nigrinus* fecundity – measured as the ratio of females to total larvae (live and dead larvae) collected per cohort – but I also examined adult mortality, differences in adult mortality based on gender, egg and

larval production, and performed genetic analyses on several PNW and NC cohort specimens in 2013 (although no genetic analyses were performed on *L. nigrinus* in 2014). Data were recorded and analyzed to determine whether there were any significant differences in fecundity between field cohorts (PNW and NC) versus F_1 cohorts (PNW-F_1 and NC-F_1), between the two field cohorts (PNW vs NC) and the two laboratory cohorts (PNW-F_1 vs NC-F_1), and whether factors other than fecundity could affect the number of larvae produced in a laboratory setting (i.e., gender distribution, adult female mortality, adult male mortality) in each cohort. The number of filial adults produced by the 2013 cohorts were also recorded but served as an indicator of the success of our laboratory rearing techniques in bringing collected larvae to maturation rather than a measure of fecundity.

In 2013, I used the numbers of males and females in each cohort to determine whether there was a difference in the rate of mortality for either gender among cohorts. Numbers of males and females were estimates based on beetles sexed at the conclusion of the study. Male and female numbers were determined post-mortem in 2013 and new beetles from reserve containers replaced dead and missing beetles. Therefore, it was not possible to determine the exact gender distributions per week in 2013. Instead, sex ratio was estimated for each week of the experiment, excluding the gender of any missing beetles. Numbers were based on the total number of beetles available at the insectary (i.e., beetles used in the experiment plus beetles held in reserve jars; all jars were sexed at the end of the study and dead and missing beetles from oviposition jars in the study were replaced

by reserve jar beetles throughout the study).

Hybridization:

In 2013, I noticed that several beetles in the NC cohort had a red tint to their elytra. Because of this, I decided to perform genetic analyses on six of the NC beetles with this phenotypic distinction to discern whether this phenotypic anomaly may be an indication of *L. nigrinus* x *L rubidus* hybridization. I based my methods on Klein et al. (2010). I was able to secure pure strain *L. nigrinus* from my PNW cohort as a baseline, but was unable to secure *L. rubidus* specimens known to be pure strain. Genetic analyses were performed under the supervision of Dr. C. Saski and X. Xia, Department of Genetics and Biochemistry, Clemson University, SC.

DNA purifications were carried out on frozen individual specimens. Whole individuals were homogenized in LN2 (liquid nitrogen) in a mortar and pestle and immediately added to extraction buffer from kit ENZA Insect DNA kit (omega Bio-Tek, D0926-01, Norcross, GA). Six NC beetles and six PNW beetles were analyzed. The six NC beetles were analyzed individually, while the PNW beetles were analyzed as two groups of three (as these beetles were known to be *L. nigrinus*). DNA was purified according to the manufacturer's recommended procedure and concentrations determined by UV Spec (Nanodrop 8000; Thermo Fisher Scientific, Inc., Biosciences, Waltham, MA). Genomic DNA was normalized to 20 ng per microliter for each individual (NC), or group of three individuals (PNW). Microsatellite loci LaGT04, LaGT07, and LaGT19 were selected for

amplification (Klein et al., 2010). Locus specific primers were ordered from EZNA insect DNA kit (50 preps) and a fluorescent dye was incorporated on the 5' end of each forward primer. PCR amplifications were performed by combining 10 ng of template, 80 uM dNTPs, 2 mM $MgCl_2$, 0.5 units of AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific, Inc., Applied Biosystems, Waltham, MA), and 0.1uM of each forward primers: LaCA04, 6fam-tcctctttacgacacatacattttg; LaGT07, vic-ggcaaatgcatgacaaggc; LaGT19, pet-tgatgcaggaagattttgacag (Fisher Scientific, Inc., Life Technologies, oligos --> Fisher Scientific, Inc., Applied biosystems, 5 primer labeled primers, 10,000 pico moles) and each reverse primer: LAC04_R, Acacgctcactggagaagg; LAG07_R, tgggttatatggatgagaccac; LAGT19_R, accaagtttgattcctcttcgac (Integrated DNA Technologies, Coralville, IA).

Thermal cycling conditions were as follows: initial denaturation 15 min. at 94^{o}C; 35 cycles of 45 seconds each at 94^{o}C; 45 seconds at 55^{o}C; one min. at 72^{o}C; and final extension of 10 min. at 72^{o}C. After amplification, fluorescently labeled PCR products were mixed with 0.3 ul of GeneScan-500 LIZ size standards (Applied Biosystems) and 10 ul Hi-Di formamide (Applied Biosystems) and separated by capillary electrophoresis on an ABI 3730xl DNA analyzer. Raw trace data was analyzed with the GeneMapper Software (Applied Biosystems). Fragment sizes were determined by GeneMapper software by comparing the individual SSR (simple sequence repeats – repeating sequences of two to five base pairs of DNA) profile to the size standard (C. Saski, personal communication, July 17, 2014).

Statistical Analyses:

Data from 2013 were analyzed, but due to having only one replication per cohort (i.e., one tent for each cohort every week) no conclusive results could be drawn. Because of this, the overall results for the 2013 pilot study were compared as overall averages and Chi-square tests to the detailed results obtained in 2014. The 2014 study was composed of eight replications per cohort and allowed me to draw conclusive results regarding fecundity of each cohort.

Fecundity (larvae per female), eggs, live larvae, total larvae (live plus dead larvae), and both female and male distributions were analyzed using a linear model with effects of cohort, time, and cohort time interaction. Normal (Gaussian) random effects of repetition and error were assumed and a two-way repeated measures analysis of variance (ANOVA) was used to analyze the model. Mean differences were compared based on Fisher's Least Significant Difference (LSD) test. Contrast analyses were run as well. Beetle mortality, including those missing, and differences in male:female ratios from Week 0 to Week 8 were analyzed using a Chi-square test. All significant differences were determined at α=.10. All data was analyzed with using SAS 9.3 software (2002-2010, SAS ® Propriety Software 9.3 (TS1M2)).

CHAPTER FOUR

RESULTS

General Trends:

In 2013 and 2014, I examined production of eggs, live larvae, total (live plus dead) larvae, and larvae per female in an effort to determine which parameter could be used in a rearing system as the best measure for fecundity. Although I counted eggs in 2013, when larvae were collected it became apparent that the egg data underestimated both the number of live larvae and total larvae present for every cohort except NC-F_1 (Table 1). In 2014, the egg data were more accurate, and indicated that egg counts represented approximately twice the numbers of live larvae and total larvae for all cohorts (Table 1). The discrepancy in egg counts between 2013 and 2014 can be attributed to the difference in the number of twigs searched for eggs. In 2013, approximately 320 twigs were searched for eggs each week, while in 2014 that number was reduced to a maximum of 64 twigs searched for eggs each week. Reducing the number of twigs searched in 2014 greatly reduced human error and improved the accuracy of the egg counts.

In 2013, the overall data for both live larvae and total larvae showed different trends among cohorts (NC>PNW-F_1>NC-F_1>PNW) than were observed for these parameters in 2014 (PNW>NC>PNW-F_1>NC-F_1) (Table 1). However, cohorts followed the same trend (PNW>NC>PNW-F_1>NC-F_1) for the average number of larvae per female both years. The PNW cohort produced the highest average number of larvae per female, followed by the NC cohort. The average larvae per female numbers for the wild-caught cohorts were

considerably higher in 2014 than in 2013. The two laboratory-reared F_1 cohorts exhibited lower average number of larvae per female than either of the wild-caught cohorts both years. The average number of larvae per female showed a difference of less than one larva per female between 2013 and 2014 for the laboratory-reared F_1 cohorts. The PNW-F_1 cohort produced more larvae per female than did the NC-F_1 cohort (Table 1).

Table 1: Overall data for Eggs, Live Larvae, Total (Live plus Dead) Larvae, and Average Larvae per Female for 2013 and 2014 for each of the four cohorts: Pacific Northwest Wild-Caught (PNW), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), and North Carolina Laboratory-Reared F_1 Generation (NC-F_1).

	Cohort	Eggs	Live Larvae	Total Larvae	Average Larvae per Female
2013	PNW	1994	2185	2271	42.6
	PNW-F_1	1923	3406	3558	35.6
	NC	2118	4095	4185	40.5
	NC-F_1	2415	2422	2584	26.5
2014	PNW	1665	830	866	59.4
	PNW-F_1	976	537	576	36.0
	NC	1277	674	729	51.0
	NC-F_1	830	413	436	27.3

Sex Ratio and Adult Mortality 2013:

At Week 0 there were 1.96 males for every female in the PNW cohort, 1.05 males for every female in the PNW-F_1 cohort, 1.08 males for every female in the NC cohort, and 1.14 males for every female in the NC-F_1 cohort (Fig. 21). The greatest change in sex ratios by the end of Week 8 was seen in the PNW cohort, which had 9.28 males for every female at the conclusion of the experiment. The other cohorts exhibited less dramatic changes in sex ratios, ending with 1.55 males per female in PNW-F_1, 1.14 males for

every female in NC, and 1.28 males for every female in NC-F_1 (Fig. 21). A chi-square test was run to determine the hypothesis: H_o: (Change of Male:Female Distribution from Week 0 to Week 8)$_{PNW}$= (Change of Male:Female Distribution from Week 0 to Week 8)$_{PNW-F1}$= (Change of Male:Female Distribution from Week 0 to Week 8)$_{NC}$= (Change of Male:Female Distribution from Week 0 to Week 8)$_{NC-F1.}$ Chi-square tests confirm that there was a significant difference in percent distribution of males to females from Week 0 to Week 8 between the following cohorts:

- PNW and PNW-F_1 ($p<.0001$);
- PNW and NC ($p<.0001$);
- PNW and NC-F_1 ($p<.0001$);
- PNW-F_1 and NC ($p=.0686$).

The following cohorts did not differ significantly from one another:

- PNW-F_1 and NC-F_1 ($p=.1291$);
- NC and NC-F_1 ($p=.7399$).

There was a higher overall loss of beetles in the PNW cohort than in any other cohort in 2013. The total number of live adults declined for all cohorts between Weeks 0 through 8. This loss was most apparent in the PNW cohort (Fig. 22).

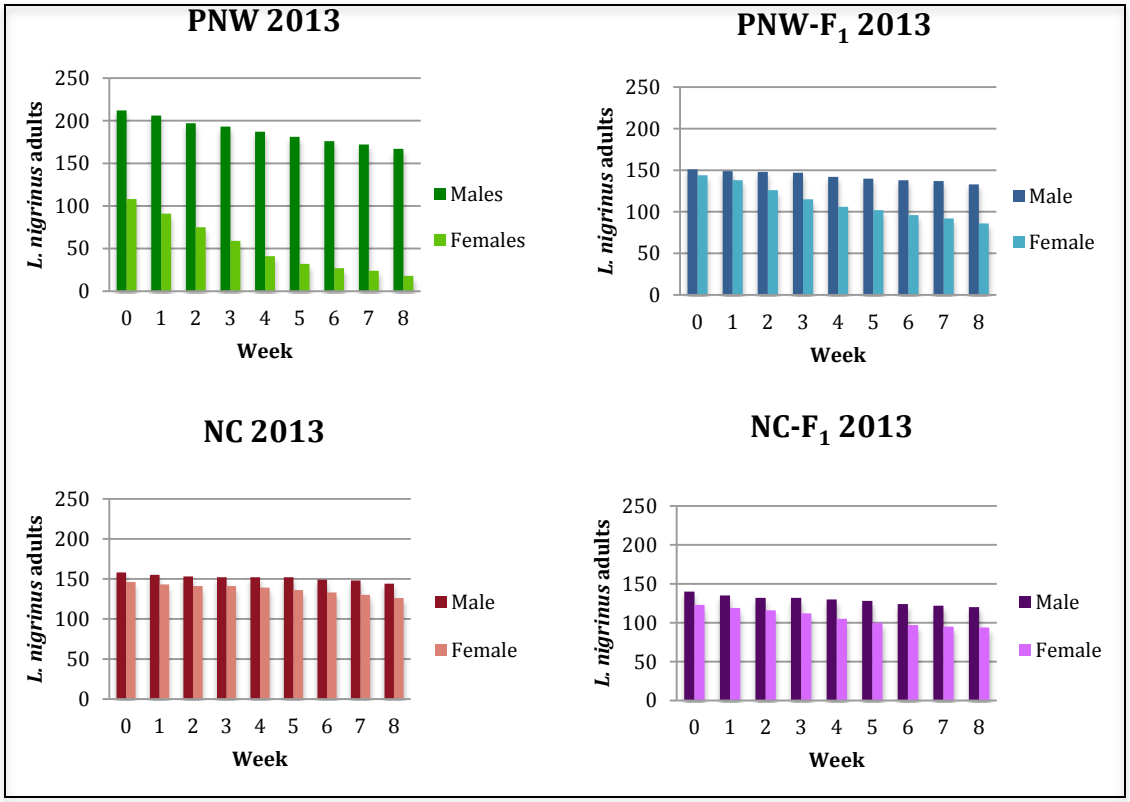

Figure 21: Estimated numbers of live *L. nigrinus* males and females in each cohort (Pacific Northwest Wild-Caught (PNW), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1)) at the end of each week in 2013. Week 0 is the week before the study began.

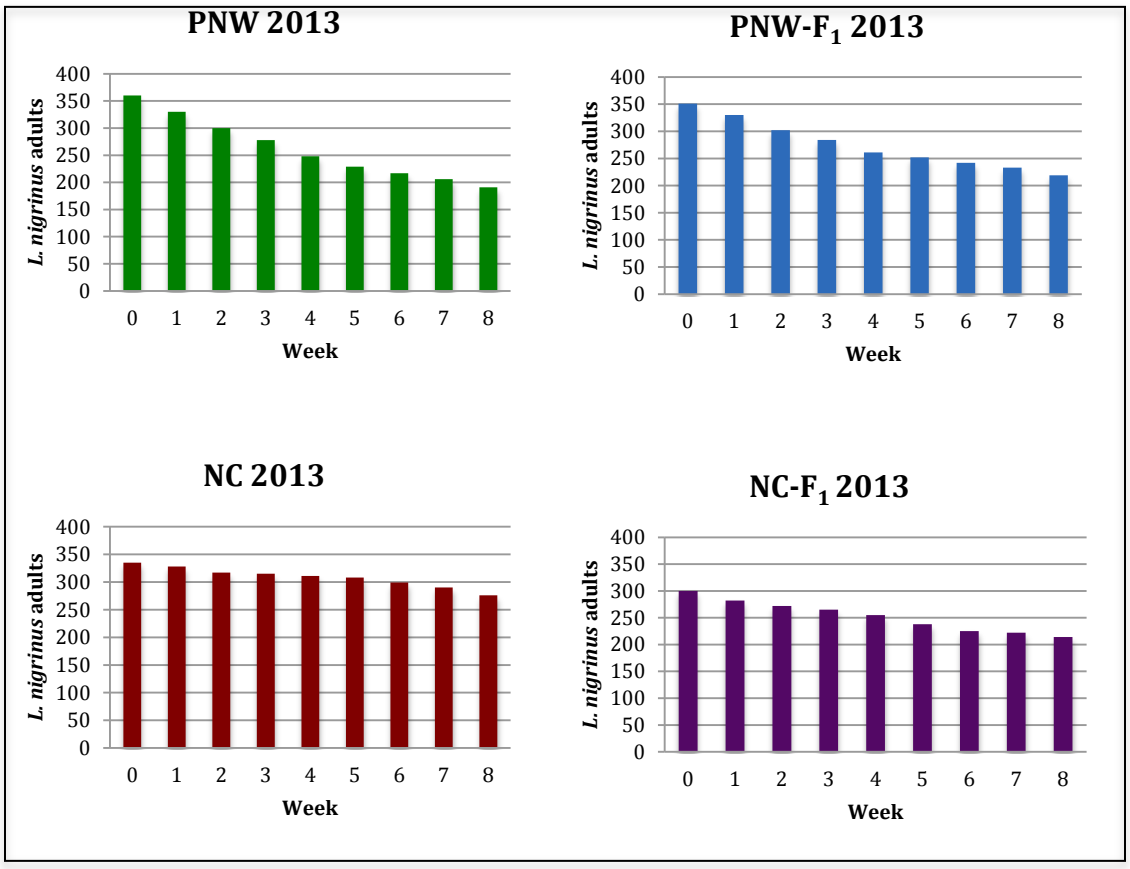

Figure 22: Live adult *L. nigrinus* per week by cohort: Pacific Northwest Wild-Caught (PNW), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), and North Carolina Laboratory-Reared F_1 Generation (NC-F_1) during the course of the 2013 study. Numbers are based on the total number of beetles available at the insectary at the end of each week (i.e., beetles used directly in the experiment and beetles held in reserve). Week 0 is the week before the study began.

With a 42.8% decline in number of beetles in oviposition jars during the study, the PNW cohort showed the greatest decline of any cohort in 2013. The PNW-F_1 cohort showed a 33.6% decline, followed by the NC-F_1 cohort, which experienced a 26.0% decline. The NC cohort exhibited the lowest loss in 2013 at 13.4%. A chi-square test was run to determine the hypothesis: H_o: $(\text{Loss of Adults})_{PNW} = (\text{Loss of Adults})_{PNW\text{-}F1} = (\text{Loss of Adults})_{NC} = (\text{Loss of Adults})_{NC\text{-}F1}$. Chi-square tests confirm that there was a significant difference in loss of beetles from Week 0 to Week 8 between all of the:

- PNW and PNW-F_1 (p=.0118);
- PNW and NC (p<.0001);
- PNW and NC-F_1 (p<.0001);
- PNW-F_1 and NC (p<.0001);
- PNW-F_1 and NC-F_1 (p=.0160);
- NC and NC-F_1 (p=.0009).

The cumulative number of dead beetles at the end of each week in 2013 is shown in Fig. 23. The PNW cohort had the highest rate of beetle mortality compared to the other cohorts. The NC cohort showed the lowest rate of beetle mortality and the lowest overall beetle mortality in 2013.

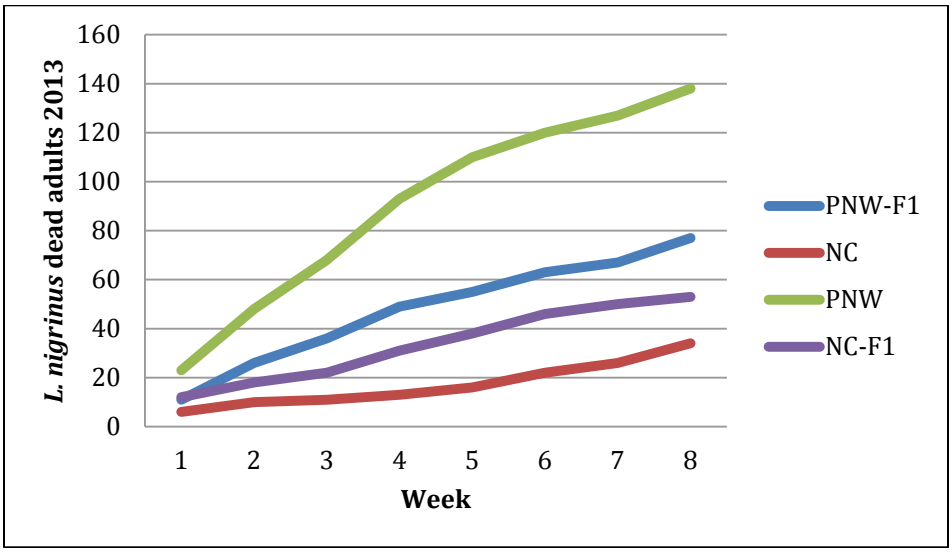

Figure 23: Weekly cumulative dead *L. nigrinus* adults collected from oviposition jars for each cohort at the conclusion of each week in 2013. Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), and North Carolina Laboratory-Reared F_1 Generation (NC-F_1).

Sex Ratio and Adult Mortality 2014:

In 2014, I began the experiment with one male for every female in each cohort (Fig. 24). The PNW cohort lost five males and five females during the eight-week study. The PNW-F_1 cohort lost two males and no females during the eight-week study, the NC cohort lost one male and three females, and the NC-F_1 cohort lost four males and zero females (Fig. 24). At the conclusion of the experiment, the sex ratios (male:female) were: PNW (1:1), PNW-F_1 (0.88:1), NC (1.15:1), and NC-F_1 (0.75:1). A chi-square test was run to determine the hypothesis: H_o: (Change of Male:Female Distribution from Week 0 to Week 8)$_{PNW}$= (Change of Male:Female Distribution from Week 0 to Week 8)$_{PNW\text{-}F1}$= (Change of Male:Female Distribution from Week 0 to Week 8)$_{NC}$= (Change of Male:Female Distribution from Week 0 to Week 8)$_{NC\text{-}F1.}$ Chi-square tests confirm that there was a significant difference in percent distribution of males to females from Week 0 to Week 8 between two cohorts:

- NC and NC-F_1 (p=.0367).

The remaining cohorts did not differ significantly from one another in changes of male to female distribution:

- PNW and PNW-F_1 (p=.5117);
- PNW and NC (p=.4773);
- PNW and NC-F_1 (p=.1666);
- PNW-F_1 and NC (p=.1712);
- PNW-F_1 and NC-F_1 (p=.4661).

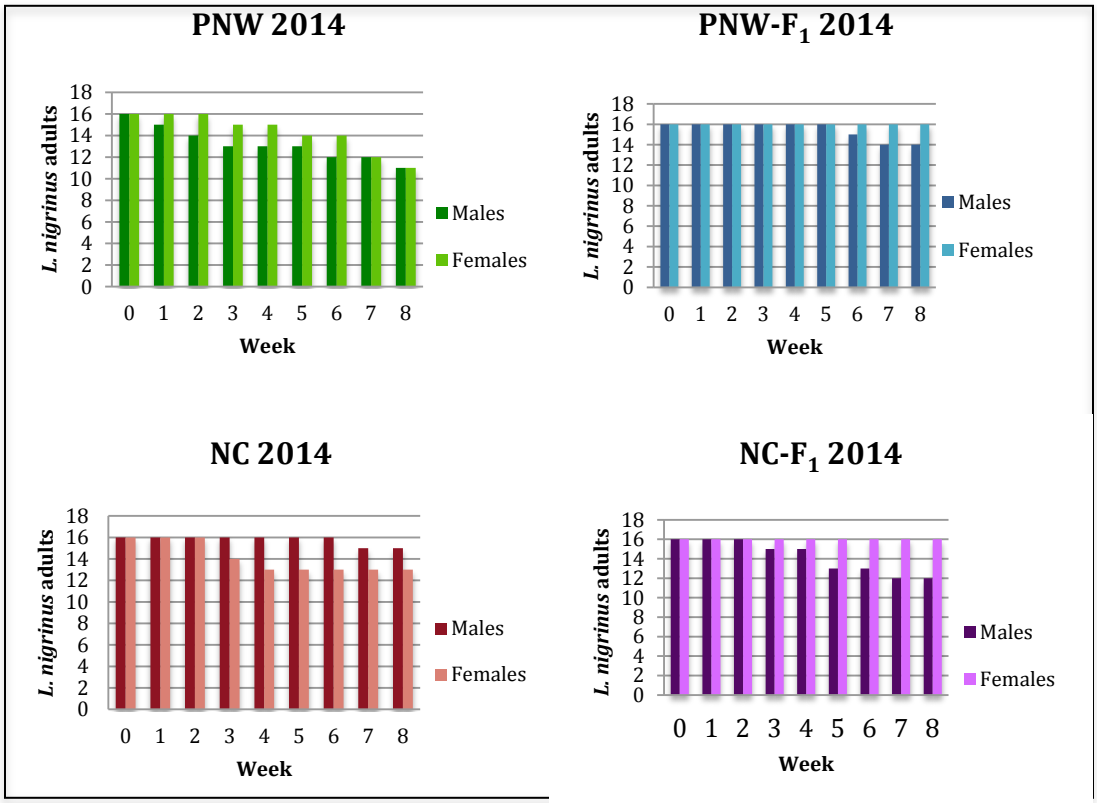

Figure 24: Number of *L. nigrinus* males and females at the beginning of each experimental week by cohort: Pacific Northwest Wild-Caught (PNW), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), for each week of the study in 2014. Week 0 is the week before the study began.

The weekly loss of adult beetles by cohort is shown in Fig. 25. By the end of Week 8, the PNW-F_1 cohort experienced the least amount of loss (6.25%) with 30 beetles remaining alive, both the NC and NC-F_1 cohorts lost four beetles (12.50%), ending the study with 28 beetles. The PNW cohort had the fewest beetles at the end of the experiment with 22 adults (31.25% loss). A chi-square test was run to determine the hypothesis: H_o: (Loss of Adults)$_{PNW}$= (Loss of Adults)$_{PNW\text{-}F1}$= (Loss of Adults)$_{NC}$= (Loss of Adults)$_{NC\text{-}F1.}$ Chi-square tests confirm that there was a significant difference in beetle loss from Week 0 to Week 8 between the following cohorts:

- PNW and PNW-F_1 (p=.0104);
- PNW and NC (p=.0696);
- PNW and NC-F_1 (p=.0696);

The following cohorts did not differ significantly from one another:

- PNW-F_1 and NC (p=.3911);
- PNW-F_1 and NC-F_1 (p=.3911);
- NC and NC-F_1 (p=1.0000).

The cumulative number of dead beetles at the end of each week in 2014 is shown in Fig. 26. As in 2013, in 2014 there was a higher rate of mortality for the PNW cohort compared to the other cohorts. In 2014, PNW was the only cohort exhibiting mortality during Week 8. In total, the PNW mortality was ten *L. nigrinus* adults. Unlike adult mortality in 2013, the order of highest mortality to lowest mortality changed for the remaining three cohorts (from PNW-F_1>NC-F_1>NC in 2013 to NC>PNW-F_1=NC-F_1 in 2014). In 2014, the NC cohort only lost three beetles. Both laboratory-reared cohorts (PNW-F_1 and NC-F_1) lost two beetles during the study.

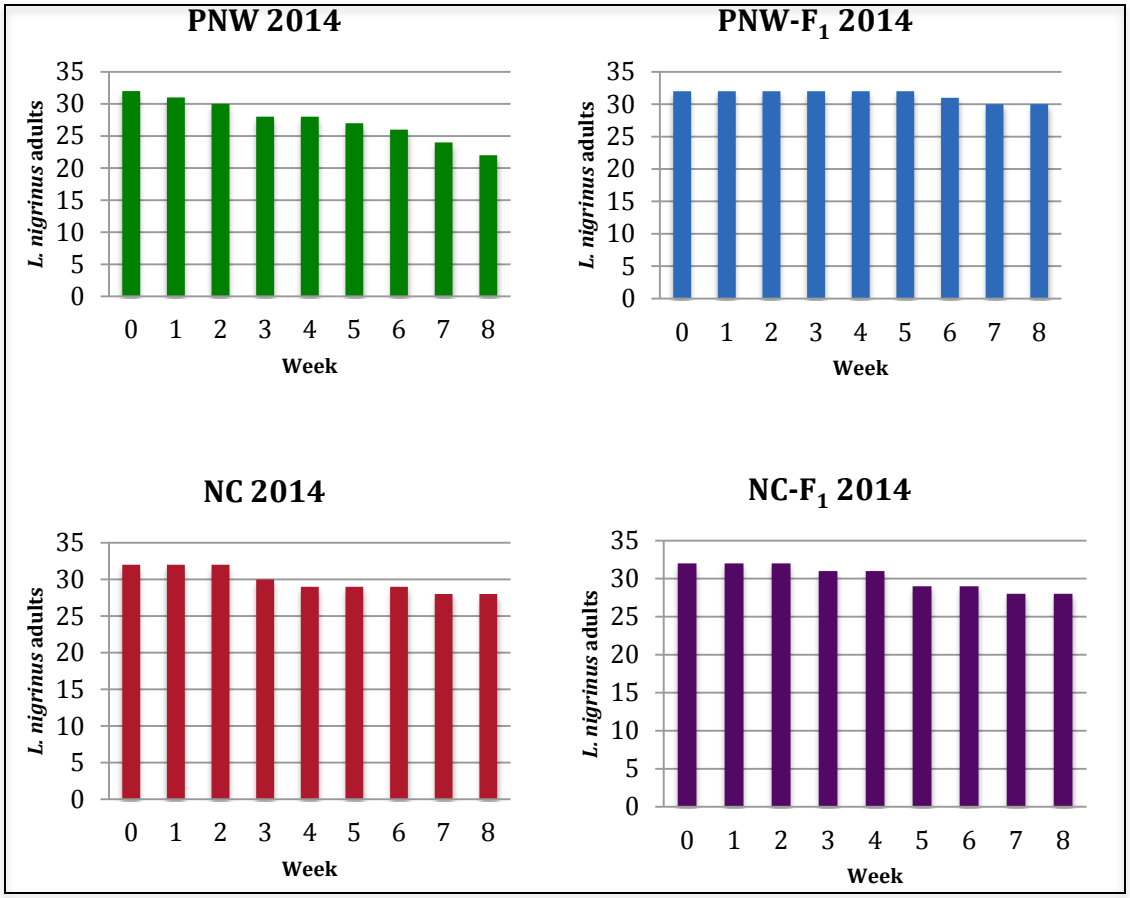

Figure 25: Number of live adult *L. nigrinus* in each cohort (Pacific Northwest Wild-Caught (PNW), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), and North Carolina Laboratory-Reared F_1 Generation (NC-F_1)), in 2014 from Week 0 through Week 8. Week 0 is the week before the study began.

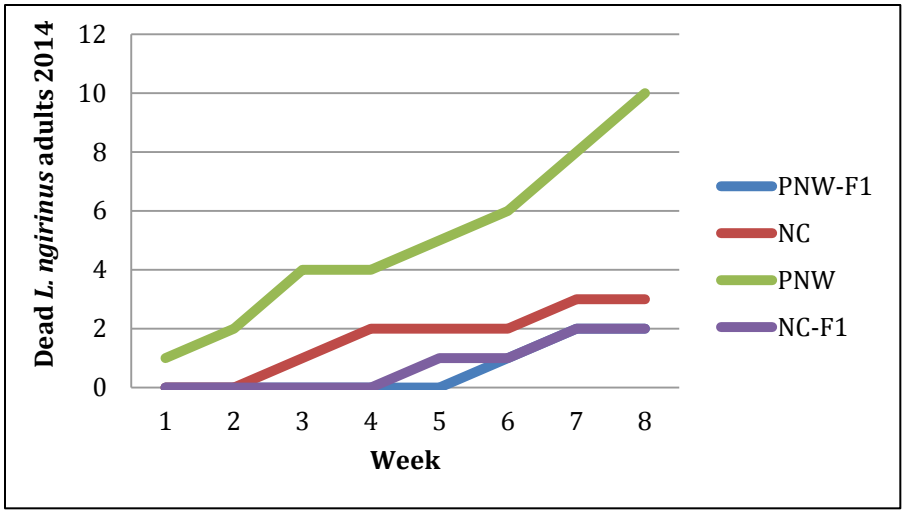

Figure 26: Cumulative total dead *L. nigrinus* adults recovered from oviposition jars for each cohort at the conclusion of each week in 2014. Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), North Carolina Wild-Caught (NC), and North Carolina Laboratory-Reared F_1 Generation (NC-F_1).

Larvae per Female 2014:

The overall and weekly least squares mean numbers of larvae per female for 2014 are shown in Table 2. There were significant differences in least squares means for larvae per female among cohorts, weeks, and the cohort*weeks interactions. The hypothesis test of significant difference in least squares means among cohorts is H_o: $\mu_{PNW} = \mu_{PNW\text{-}F1} = \mu_{NC} = \mu_{NC\text{-}F1}$. The type III tests of fixed effects for least squares means larvae per female resulted in a p-value of .0016. I further analyzed the differences among cohorts in mean terms of larvae per female with Fisher's LSD test (Table 2). Cohorts that differed significantly from one another for this parameter were:

- PNW > PNW-F_1 (p=.0065);
- PNW > NC-F_1 (p=.0004);

- NC > PNW-F_1 (p=.0704);
- NC > NC-F_1 (p=.0059).

The PNW and NC cohorts were not significantly different from one another (p=.2991). Likewise, the PNW-F_1 and NC-F_1 cohorts were not significantly different from one another (p=.2819) (Table 2).

Table 2: Least squares means and standard errors for *L. nigrinus* larvae per female for each of the four cohorts in 2014: North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1). Within weeks, means with the same letter are not significantly different based on Fisher's LSD test.

Larvae per Female								
	PNW		PNW-F_1		NC		NC-F_1	
	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Means	Standard Error
Week 1	9.3125[a]	1.0509	5.5625[b]	1.0509	5.5625[b]	1.0509	4.2500[b]	1.0509
Week 2	8.2500[ab]	1.3840	6.6250[b]	1.3840	9.5625[a]	1.3840	6.1875[b]	1.3840
Week 3	9.2500[a]	1.1311	6.4375[b]	1.1311	6.6875[ab]	1.1311	4.3750[b]	1.1311
Week 4	11.2500[a]	1.7040	4.2500[b]	1.7040	8.8750[a]	1.7040	3.7500[b]	1.7040
Week 5	8.3750[a]	1.1972	4.2500[b]	1.1972	9.9375[a]	1.1972	3.1875[b]	1.1972
Week 6	6.5000[a]	1.0165	2.9375[b]	1.0165	3.8750[b]	1.0165	2.8750[b]	1.0165
Week 7	5.0625[a]	0.6754	3.5000[a]	0.6754	3.7500[a]	0.6754	1.3125[b]	0.6754
Week 8	1.4375[ab]	0.5705	2.4375[ab]	0.5705	2.7500[a]	0.5705	1.3125[b]	0.5705
Overall	7.4297[a]	0.7049	4.5000[b]	0.7049	6.3750[a]	0.7049	3.4062[b]	0.7049

The hypothesis test of significant difference in least squares means among weeks is H_o: $\mu_{Week1} = \mu_{Week2} = \mu_{Week3} = \mu_{Week4} = \mu_{Week5} = \mu_{Week6} = \mu_{Week7} = \mu_{Week8}$. The type III tests of fixed effects for larvae per female indicated that there was a significant difference among weeks ($p<.0001$). The least squares means for larvae per female for individual weeks were: Week 1 ($6.1719^{b}\pm0.5715$); Week 2 ($7.6563^{a}\pm0.5715$) Week 3 ($6.6875^{ab}\pm0.5715$); Week 4 ($7.0313^{ab}\pm0.5715$); Week 5 ($6.4375^{b}\pm0.5715$); Week 6 ($4.0469^{c}\pm0.5715$); Week 7 ($3.4063^{c}\pm0.5715$); Week 8 ($1.9844^{d}\pm0.5715$) (means with the same letter are not significantly different based on Fisher's LSD test). The following adjacent weeks differed significantly from one another in least squares means larvae per female:

- Week 1 < Week 2 (p=.0303);
- Week 5 > Week 6 (p=.0005);
- Week 7 > Week 8 (p=.0378).

The pattern of least squares means for larvae per female during the 8-week study showed a decline over time for larvae per female for each cohort. The PNW cohort consistently had the highest least squares mean for larvae per female except in two weeks when the NC cohort had higher values (Weeks 2 & 5). However, in both of these weeks this difference was not statistically significant.

In 2014, according to the type III test for fixed effects for larvae per female, there was a significant cohort*weeks interaction for larvae per female. The hypothesis test of significant difference in weekly change among cohorts is H_o: $(\text{Weekly Change})_{PNW} =$

$(\text{Weekly Change})_{\text{PNW-F1}} = (\text{Weekly Change})_{\text{NC}} = (\text{Weekly Change})_{\text{NC-F1}}$. The p-value (.0025) indicated that a significant interaction existed.

Comparison of least squares means of *L. nigrinus* larvae per female showed that wild-caught cohorts combined > laboratory-reared cohorts combined (p=.0003) (Table 4: Wild vs Laboratory). Comparison of the overall least squares means of *L. nigrinus* larvae per female for the PNW cohort versus the combined data from the NC, PNW-F_1, and NC-F_1 cohorts indicated significant differences in the least squares means (p=.0028) where Western>Eastern (Table 4: Western vs Eastern).

Table 3: Cohort contrasts of *L. nigrinus* larvae per female. "Wild" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) and the North Carolina Wild-Caught (NC) cohorts, while "Laboratory" represents the least squares mean of the Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1) and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts. "Western" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) cohort while "Eastern" represents the least squares mean of the combined remaining cohorts: North Carolina (NC), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts.

Larvae per Female Contrasts				
Label	Least Squares Mean		t Value	Pr > F
Wild vs Laboratory	Wild 6.9024±0.7049	Laboratory 3.9531±0.7049	4.18	0.0003
Western vs Eastern	Western 7.4297±0.8140	Eastern 4.7604±0.8140	3.28	0.0028

Live Larvae 2014:

The overall and weekly least squares mean numbers of live larvae for 2014 are shown in Table 4. There were significant differences of least square means in 2014 for live larvae among cohorts, weeks, and cohort*weeks. The hypothesis test of significant difference for least squares means among cohorts is H_o: $\mu_{PNW} = \mu_{PNW\text{-}F1} = \mu_{NC} = \mu_{NC\text{-}F1}$. The type III test for fixed effects for live larvae resulted in a p-value of .0021. The number of live larvae produced in 2014 indicate that the following relationships existed among cohorts: PNW > PNW-F_1 (p=.0072); PNW > NC-F_1 (p=.0003); and NC> NC-F_1 (p=.0153) (Table 4). The NC cohort did not differ significantly in least squares means of live larvae from the PNW-F_1 cohort (p=.1858). The least square means of live larvae, were not significantly different between either the PNW and NC cohorts (p=.1337), or the PNW-F_1 and NC-F_1 cohorts (p=.2298) (Table 4).

Table 4: Least squares means and standard errors for *L. nigrinus* live larvae in each of the four cohorts in 2014: North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1). Within weeks, means with the same letter are not significantly different based on Fisher's LSD test.

Live Larvae								
	PNW		PNW-F_1		NC		NC-F_1	
	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error
Week 1	17.8750^{a}	2.1471	11.0000^{b}	2.1471	10.6250^{b}	2.1471	8.3750^{b}	2.1471
Week 2	16.8750^{ab}	2.2471	10.6250^{c}	2.2471	17.7500^{a}	2.2471	11.8750^{bc}	2.2471
Week 3	16.8750^{a}	2.1771	11.8750^{ab}	2.1771	11.5000^{b}	2.1771	8.0000^{b}	2.1771
Week 4	18.2500^{a}	1.9235	8.2500^{c}	1.9235	13.1250^{b}	1.9235	6.1250^{c}	1.9235
Week 5	14.0000^{a}	1.9415	7.8750^{b}	1.9415	14.5000^{a}	1.9415	6.2500^{b}	1.9415
Week 6	9.7500^{a}	1.4659	5.8750^{b}	1.4659	6.2500^{ab}	1.4659	5.7500^{b}	1.4659
Week 7	8.1250^{a}	1.2540	6.7500^{a}	1.2540	6.5000^{a}	1.2540	2.6250^{b}	1.2540
Week 8	2.0000^{b}	0.9791	4.8750^{a}	0.9791	4.0000^{ab}	0.9791	2.6250^{ab}	0.9791
Overall	12.9688^{a}	1.1169	8.3906^{bc}	1.1169	10.5313^{ab}	1.1169	6.4531^{c}	1.1169

The hypothesis test of significant difference in least squares means among weeks is H_o: $\mu_{Week1} = \mu_{Week2} = \mu_{Week3} = \mu_{Week4} = \mu_{Week5} = \mu_{Week6} = \mu_{Week7} = \mu_{Week8}$. The type III tests of fixed effects for live larvae resulted in a p-value of <.0001. The least squares means for live larvae for individual weeks were: Week 1 ($11.9688^{b} \pm 0.9109$); Week 2

(14.2813[a]±0.9109); Week 3 (12.0625[b]±0.9109); Week 4 (11.4375[b]±0.9109); Week 5 (10.6563[b]±0.9109); Week 6 (6.9063[c]±0.9109); Week 7 (6.0000[c]±0.9109); Week 8 (3.3750[d]±0.9109) (means with the same letter are not significantly different based on Fisher's LSD test). The following adjacent weeks differed significantly from one another in least squares means of larvae per female:

- Week 1 < Week 2 (p=.0349);
- Week 2 > Week 3 (p=.0428);
- Week 5 > Week 6 (p=.0007);
- Week 7 > Week 8 (p=.0168).

Although not significant, the least squares means of live larvae were higher for the NC cohort in Weeks 2 and 5 than in the PNW cohort. In Week 8, the PNW cohort had the lowest least squares mean of live larvae of any cohort, with a significant difference occurring between the PNW (2.0000) and the PNW-F_1 (4.8750) cohorts (p=.0472) (Table 4).

In 2014, according to the type III test for fixed effects for larvae per female, there was a significant cohort*weeks interaction for live larvae (p=.0018). The hypothesis test of significant interaction is H_o: $(\text{Weekly Change})_{PNW} = (\text{Weekly Change})_{PNW\text{-}F1} = (\text{Weekly Change})_{NC} = (\text{Weekly Change})_{NC\text{-}F1}$.

Comparisons of least squares means of *L. nigrinus* live larvae for the wild-caught cohorts combined versus the laboratory-reared cohorts combined indicated that the least squares mean for live larvae of wild-caught cohorts was significantly greater than the least squares mean for the laboratory cohorts (p=.0006) (Table 5: Wild vs Laboratory). Comparison of the overall least squares means of *L. nigrinus* live larvae for the PNW cohort versus the combined data from the NC, PNW-F_1, and NC-F_1 cohorts showed a that Western>Eastern (p=.0016) (Table 5: Western vs Eastern).

Table 5: Cohort contrasts of *L. nigrinus* live larvae. "Wild" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) and the North Carolina Wild-Caught (NC) cohorts, while "Laboratory" represents the least squares mean of the Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1) and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts. "Western" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) while "Eastern" represents the least squares mean of the combined remaining cohorts: North Carolina (NC), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts.

Live Larvae Contrasts				
Label	Least Squares Mean		t Value	Pr > F
Wild vs Laboratory	Wild 11.7501±1.1160	Laboratory 7.4219±1.1160	3.88	0.0006
Western vs Eastern	Western 12.9688±1.2886	Eastern 8.4583±1.2886	3.50	0.0016

Total Larvae 2014:

The differences of least square means for *L. nigrinus* total (live plus dead) larvae among cohorts, weeks, and cohort*weeks is shown in Table 6. There is evidence of significant differences of least square means in 2014 for total (live plus dead) larvae among cohorts, weeks, and cohort*weeks interactions. The hypothesis test of significant difference in least squares means among cohorts is H_o: $\mu_{PNW} = \mu_{PNW\text{-}F1} = \mu_{NC} = \mu_{NC\text{-}F1}$. The type III tests of fixed effects for total larvae resulted in a p-value of .0024. Results for the total number of *L. nigrinus* larvae produced in the larval rearing boxes in 2014 show the PNW > PNW-F_1 (p=.0108); PNW > NC-F_1 (p=.0004); and NC > NC-F_1 (p=.0101). The NC cohort did not show a significant difference compared to the PNW-F_1 cohort (p=.1607). The two wild-caught cohorts (PNW and NC) were not significantly different from one another (p=.2075). The two laboratory reared cohorts (PNW-F_1 and NC-F_1) were also not significantly different from one another (p=.1980) (Table 6).

Table 6: Least squares means and standard errors for *L. nigrinus* total (live plus dead) larvae in each of the four cohorts in 2014: North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1). Within weeks, means with similar letters are not significantly different based on Fisher's LSD test.

Total Larvae								
	PNW		PNW-F_1		NC		NC-F_1	
	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error
Week 1	18.6250^{a}	2.1019	11.1250^{b}	2.1019	11.1250^{b}	2.1019	8.5000^{b}	2.1019
Week 2	16.5000^{ab}	2.7680	13.2500^{ab}	2.7680	19.1250^{a}	2.7680	12.3750^{b}	2.7680
Week 3	18.5000^{a}	2.2621	12.8750^{b}	2.2621	13.3750^{ab}	2.2621	8.7500^{b}	2.2621
Week 4	18.6250^{a}	1.9108	8.5000^{b}	1.9108	14.5000^{a}	1.9108	7.5000^{b}	1.9108
Week 5	14.7500^{a}	2.0683	8.5000^{b}	2.0683	15.8750^{a}	2.0683	6.3750^{b}	2.0683
Week 6	10.5000^{a}	1.4914	5.8750^{b}	1.4914	6.3750^{b}	1.4914	5.7500^{b}	1.4914
Week 7	8.5000^{a}	1.2640	7.0000^{a}	1.2640	6.5000^{a}	1.2640	2.6250^{b}	1.2640
Week 8	2.2500^{b}	0.9769	4.8750^{a}	0.9769	4.2500^{ab}	0.9769	2.6250^{ab}	0.9769
Overall	13.5312^{a}	1.1732	9.0000^{bc}	1.1732	11.3906^{ab}	1.1732	6.8125^{c}	1.1732

The hypothesis test of significant difference in least squares means among weeks is H_o: $\mu_{Week1} = \mu_{Week2} = \mu_{Week3} = \mu_{Week4} = \mu_{Week5} = \mu_{Week6} = \mu_{Week7} = \mu_{Week8}$. The type III tests of fixed effects for total larvae for the weeks indicated that there was a significant difference among weeks ($p<.0001$). The least squares means for total larvae for individual weeks were: Week 1 ($12.3438^{bc} \pm 0.9667$); Week 2 ($15.3125^{a} \pm 0.9667$); Week 3

(13.3750[b]±0.9667); Week 4 (12.2813[bc]±0.9667); Week 5 (11.3750[c]±0.9667); Week 6 (7.1250[d]±0.9667); Week 7 (6.1563[d]±0.9667); Week 8 (3.5000[e]±0.9667) (means with the same letter are not significantly different based on Fisher's LSD test). The following adjacent weeks differed significantly from one another in least squares means of larvae per female:

- Week 1 < Week 2 (p=.0114);
- Week 2 > Week 3 (p=.0969);
- Week 5 > Week 6 (p=.0003);
- Week 7 > Week 8 (p=.0233).

The least squares means of total larvae were higher for the NC cohort in Weeks 2 and 5 than the PNW cohort, though these differences were not significant. By Week 8, the PNW cohort had the lowest least squares mean of total larvae out of any cohort. In Week 8 there was a significant difference between the least squares means of total larvae for PNW<PNW-F1 (p=.0678) (Table 6).

In 2014, according to the type III test for fixed effects for total larvae there is evidence of a significant cohort*weeks combination for total larvae. The hypothesis test of significant interaction is H_o: $(\text{Weekly Change})_{PNW} = (\text{Weekly Change})_{PNW\text{-}F1} = (\text{Weekly Change})_{NC} = (\text{Weekly Change})_{NC\text{-}F1}$. The p-value for the Cohort*Weeks interaction indicates that there is a significant difference in weekly change among the four cohorts (p=.0057) .

Comparison of least squares means of *L. nigrinus* total (live and dead) larvae for the wild-caught cohorts combined versus the laboratory-reared cohorts combined indicated a significant differences in the least squares mean with Wild > Laboratory (p=.0006) (Table 7: "Wild vs Laboratory"). Comparison of the least squares mean of *L. nigrinus* total larvae for the PNW cohort versus the combined data from the NC, PNW-F_1, and NC-F_1 cohorts indicated significant differences in the least squares means with Western > Eastern (p=.0027) (Table 7: "Western vs Eastern").

Table 7: Cohort contrasts of *L. nigrinus* total (live plus dead) larvae. "Wild" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) and the North Carolina Wild-Caught (NC) cohorts, while "Laboratory" represents the least squares mean of the Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1) and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts. "Western" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) while "Eastern" represents the least squares mean of the combined remaining cohorts: North Carolina (NC), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts.

Total Larvae Contrasts				
Label	Least Squares Mean		t Value	Pr > F
Wild vs Laboratory	Wild 12.4609±1.1732	Laboratory 7.90625±1.1732	3.88	0.0006
Western vs Eastern	Western 13.5312±1.3547	Eastern 9.0677±1.3547	3.29	0.0027

Eggs 2014:

The overall and weekly least squares mean numbers of counted eggs for 2014 are shown in Table 8. There was evidence of significant differences of least squares means in 2014 for eggs counted among cohorts and weeks, but not for cohort*weeks combinations. The hypothesis test of significant difference in least squares means among cohorts is H_o: $\mu_{PNW} = \mu_{PNW\text{-}F1} = \mu_{NC} = \mu_{NC\text{-}F1}$. The cohort effect indicates that there is a significant difference among the cohorts ($p<.0001$).

The PNW cohort had the highest least squares mean of eggs counted of any cohort for all eight weeks (Table 8). Egg counts showed a significant difference between the two wild-caught cohorts with PNW > NC ($p=.0219$) (Table 8). The other comparisons for egg production indicated that PNW> PNW-F_1 ($p=.0002$); PNW > NC-F_1 ($p<.0001$); NC> PNW-F_1 ($p=.0701$); and NC > NC-F_1 ($p=.0092$). The two laboratory reared cohorts (PNW-F_1 and NC-F_1) were not significantly different from one another ($p=.3688$) (Table 8).

Table 8: Least squares means and standard errors for *L. nigrinus* eggs (and larvae found on twigs at time of egg count) in each of the four cohorts in 2014: North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1). Means with similar letters are not significantly different across that week on Fisher's LSD test.

Eggs								
	PNW		PNW-F_1		NC		NC-F_1	
	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error
Week 1	36.0000^{a}	2.416	20.5000^{bc}	2.416	27.2500^{b}	2.416	18.000^{c}	2.416
Week 2	28.3750^{a}	2.4862	18.6250^{b}	2.4862	28.1250^{a}	2.4862	16.0000^{b}	2.4682
Week 3	33.6250^{a}	3.1394	20.0000^{bc}	3.1394	24.3750^{b}	3.1394	15.0000^{c}	3.1394
Week 4	28.1250^{a}	2.4447	13.1250^{b}	2.4447	18.7500^{b}	2.4447	12.7500^{b}	2.4447
Week 5	26.7500^{a}	3.1687	12.6250^{b}	3.1687	15.3750^{b}	3.1687	12.3750^{b}	3.1687
Week 6	17.7500^{a}	2.6764	12.5000^{a}	2.5764	17.6250^{a}	2.5764	13.2500^{a}	2.5764
Week 7	22.7500^{a}	2.5537	13.1250^{b}	2.5537	16.1250^{ab}	2.5537	10.2500^{b}	2.5537
Week 8	14.7500^{a}	2.9142	11.5000^{ab}	2.9142	12.000^{ab}	2.9142	6.1250^{b}	2.9142
Overall	26.0156^{a}	1.766	15.2500^{c}	1.766	19.9531^{b}	1.766	12.9687^{c}	1.766

The hypothesis test of significant difference in least squares means among weeks is H_o: $\mu_{Week1} = \mu_{Week2} = \mu_{Week3} = \mu_{Week4} = \mu_{Week5} = \mu_{Week6} = \mu_{Week7} = \mu_{Week8}$. The type III tests of fixed effects for eggs resulted in $p<.0001$. The least squares means for eggs for individual weeks were: Week 1 ($25.4375^{a} \pm 1.3641$); Week 2 ($22.7813^{b} \pm 1.3641$); Week 3 ($23.2500^{ab} \pm 1.3641$); Week 4 ($18.1875^{c} \pm 1.3641$); Week 5 ($16.7813^{cd} \pm 1.3641$); Week 6

(15.2813^{d}±1.3641); Week 7 (15.5625^{d}±1.3641); Week 8 (11.0938^{e}±1.3641) (means with the same letter are not significantly different based on Fisher's LSD test). The following adjacent weeks differed significantly from one another in least squares means of eggs:

- Week 1 > Week 2 (p=.0927);
- Week 3 > Week 4 (p=.0015);
- Week 7 > Week 8 (p=.0049).

In 2014, according to the type III test for fixed effects for eggs, there was evidence of a possibly significant difference of least squares means for eggs in cohort*weeks combinations (p=.1088). The hypothesis test of significant difference in weekly change among cohorts is H_o: $(\text{Weekly Change})_{PNW} = (\text{Weekly Change})_{PNW\text{-}F1} = (\text{Weekly Change})_{NC} = (\text{Weekly Change})_{NC\text{-}F1}$.

Comparison of least squares means of *L. nigrinus* counted eggs for the wild-caught cohort combined versus the laboratory-reared cohorts combined indicated significant differences in the least squares means (p<.0001) with Wild > Laboratory (Table 9: "Wild vs Laboratory"). Comparison of the least squares means of *L. nigrinus* counted eggs for the PNW cohort versus the combined data from the NC, PNW-F_1, and NC-F_1 cohorts indicated significant differences in the least squares mean (p<.0001) with Western > Eastern (Table 9: "Western vs Eastern").

Table 9: Cohort contrasts of *L. nigrinus* eggs. "Wild" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) and the North Carolina Wild-Caught (NC) cohorts, while "Laboratory" represents the least squares mean of the Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1) and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts. "Western" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) while "Eastern" represents the least squares mean of the combined remaining cohorts: North Carolina (NC), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts.

Egg Contrasts				
Label	Least Squares Mean		t Value	Pr > F
Wild vs Laboratory	Wild 22.9843±1.7660	Laboratory 14.1093±1.7660	5.03	<.0001
Western vs Eastern	Western 26.0156±2.0392	Eastern 16.0572±2.0392	4.88	<.0001

Males 2014:

The overall and weekly least squares means of live males for 2014 are shown in Table 10. There were significant difference for adult males among weeks in 2014, but not among cohorts or in cohort*weeks interactions.

The hypothesis test of significant difference in least squares means among cohorts is H_o: $\mu_{PNW} = \mu_{PNW\text{-}F1} = \mu_{NC} = \mu_{NC\text{-}F1}$. The type III test of fixed effects for live males resulted in a p-value of .1515. Although Fisher's LSD test indicated that overall male survival was greater for the NC than the PNW cohort (p=.0431) and was greater for PNW-F_1 than for PNW (p=.0684) (Table 10). However, in order to protect against making a Type I Error (determining cohort effects are different although they are not), I chose to take the type

III test determination as the correct analysis. There were no significant differences between any of the other cohorts based on Fisher's LSD test (Table 10).

Table 10: Least squares means and standard errors for live adult males in each of the four cohorts in 2014: North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1). Means with similar letters are not significantly different across that week on Fisher's LSD test.

Live Adult Males								
	PNW		PNW-F_1		NC		NC-F_1	
	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error
Week 1	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0
Week 2	1.8750^{a}	0.08839	2.0000^{a}	0.08839	2.0000^{a}	0.08839	2.0000^{a}	0.08839
Week 3	1.7500^{b}	0.08183	2.0000^{a}	0.08183	2.0000^{a}	0.08183	2.0000^{a}	0.08183
Week 4	1.6250^{b}	0.1456	2.0000^{a}	0.1456	2.0000^{a}	0.1456	1.8750^{ab}	0.1456
Week 5	1.6250^{b}	0.1456	2.0000^{a}	0.1456	2.0000^{a}	0.1456	1.8750^{ab}	0.1456
Week 6	1.6250^{a}	0.1602	2.0000^{a}	0.1602	2.0000^{a}	0.1602	1.6250^{a}	0.1602
Week 7	1.5000^{b}	0.1736	1.8750^{ab}	0.1736	2.0000^{a}	0.1736	1.6250^{ab}	0.1736
Week 8	1.5000^{a}	0.1934	1.7500^{a}	0.1934	1.8750^{a}	0.1934	1.5000^{a}	0.1934
Overall	1.6875^{b}	0.1401	1.9531^{a}	0.1401	1.9844^{a}	0.1401	1.8125^{ab}	0.1401

The hypothesis test of significant difference in least squares means among weeks is H_o: $\mu_{Week1} = \mu_{Week2} = \mu_{Week3} = \mu_{Week4} = \mu_{Week5} = \mu_{Week6} = \mu_{Week7} = \mu_{Week8}$. The type III tests of fixed effects for live adult males indicated there was a significant difference among weeks (<.0001). The least squares means for live adult males for individual weeks were: Week 1 ($2.0000^{a}\pm0.0676$); Week 2 ($1.9688^{ab}\pm0.0676$); Week 3 ($1.9375^{ab}\pm0.0676$); Week 4 ($1.8750^{bc}\pm0.0676$); Week 5 ($1.8750^{bc}\pm0.0676$); Week 6 ($1.8125^{cd}\pm0.0676$); Week 7 ($1.7500^{de}\pm0.0676$); Week 8 ($1.6562^{e}\pm0.0676$) (means with the same letter are not significantly different based on Fisher's LSD test). There were no significant differences between adjacent weeks for live adult males in 2014.

In 2014, there is no evidence to suggest a significant difference of least squares means for live adult males in cohort*weeks combinations (p=4879). The hypothesis test of significant difference in weekly change among cohorts is H_o: $(\text{Weekly Change})_{PNW} = (\text{Weekly Change})_{PNW\text{-}F1} = (\text{Weekly Change})_{NC} = (\text{Weekly Change})_{NC\text{-}F1}$.

Comparison of least squares means of live *L. nigrinus* adult males for the wild-caught cohorts combined versus the laboratory-reared cohorts combined indicated no significant difference in the least squares means (p=.6398) (Table 11: "Wild vs Laboratory"). Comparison of the least squares means of *L. nigrinus* adult males for the PNW cohort versus the combined data from the NC, PNW-F_1, and NC-F_1 cohorts indicated a significant difference in the least squares means (p=.0549) with Eastern > Western (Table 11: "Western vs Eastern").

Table 11: Cohort contrasts of *L. nigrinus* live adult males. "Wild" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) and the North Carolina Wild-Caught (NC) cohorts, while "Laboratory" represents the least squares mean of the Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1) and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts. "Western" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) while "Eastern" represents the least squares mean of the combined remaining cohorts: North Carolina (NC), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts.

Live Adult Males Contrasts				
Label	Least Squares Mean		t Value	Pr > F
Wild vs Laboratory	Wild 1.8359±0.0991	Laboratory 1.8828±0.0991	-0.47	0.6398
Western vs Eastern	Western 1.6875±0.1144	Eastern 1.9166±0.1144	-2.00	0.0549

Females 2014:

The overall and weekly least squares means of live females for 2014 are shown in Table 12. There was evidence of a significant differences of least squares means in 2014 for adult females among cohorts, weeks, and in cohort*weeks combinations. The hypothesis test of significant difference in least squares means among cohorts is H_o: $\mu_{PNW} = \mu_{PNW\text{-}F1} = \mu_{NC} = \mu_{NC\text{-}F1}$. The type III test of fixed effects for live females resulted in a p-value of .0628. Fisher's LSD test indicated that the least squares mean of females surviving overall was greater for both the NC-F_1 (p=.0293) and PNW-F_1 (p=.0293) cohorts than the NC cohort. There were no significant differences in other cohort comparisons (Table 12).

Table 12: Least squares means and standard errors for *L. nigrinus* live adult females in each of the four cohorts in 2014: North Carolina Wild-Caught (NC), North Carolina Laboratory-Reared F_1 Generation (NC-F_1), Pacific Northwest Wild-Caught (PNW), and Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1). Means with similar letters are not significantly different across that week on Fisher's LSD test.

Live Adult Females								
	PNW		PNW-F_1		NC		NC-F_1	
	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error	Least Squares Mean	Standard Error
Week 1	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0
Week 2	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0
Week 3	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0	2.0000^{a}	0
Week 4	1.8750^{a}	0.1030	2.0000^{a}	0.1030	1.7500^{a}	0.1030	2.0000^{a}	0.1030
Week 5	1.8750^{ab}	0.1108	2.0000^{a}	0.1108	1.6250^{b}	0.1108	2.0000^{a}	0.1108
Week 6	1.7500^{ab}	0.1227	2.0000^{a}	0.1227	1.6250^{b}	0.1227	2.0000^{a}	0.1227
Week 7	1.7500^{ab}	0.1227	2.0000^{a}	0.1227	1.6250^{b}	0.1227	2.0000^{a}	0.1227
Week 8	1.5000^{b}	0.1315	2.0000^{a}	0.1315	1.6250^{b}	0.1315	2.0000^{a}	0.1315
Overall	1.8438^{ab}	0.0673	2.0000^{a}	0.0673	1.7813^{b}	0.0673	2.0000^{a}	0.0673

The hypothesis test of significant difference in least squares means among weeks is H_o: $\mu_{Week1} = \mu_{Week2} = \mu_{Week3} = \mu_{Week4} = \mu_{Week5} = \mu_{Week6} = \mu_{Week7} = \mu_{Week8}$. The type III tests of fixed effects for live adult females for weeks resulted in a p-value of <.0001. The least squares means for live adult females for individual weeks were: Week 1 ($2.0000^{a} \pm 0.0469$); Week

2 (2.0000^{a}±0.0469); Week 3 (2.0000^{a}±0.0469); Week 4 (1.9063^{b}±0.0469); Week 5 (1.8750^{b}±0.0469); Week 6 (1.8438^{bc}±0.0469); Week 7 (1.8438^{bc}±0.0469); Week 8 (1.7813^{c}±0.0469) (means with the same letter are not significantly different based on Fisher's LSD test). There was a significant difference between Week 3 > Week 4 (p=.0587) for live adult females in 2014.

In 2014, there was evidence to suggest a significant difference of least squares means for live adult females in cohort*weeks interactions. The hypothesis test of significant differences in weekly change among cohorts is H_o: $(\text{Weekly Change})_{PNW} = (\text{Weekly Change})_{PNW\text{-}F1} = (\text{Weekly Change})_{NC} = (\text{Weekly Change})_{NC\text{-}F1}$. There was a significant cohort*week effect among the four cohorts for live adult females (p=.0007).

Least squares means of *L. nigrinus* adult females for the wild-caught cohorts combined (1.8125±0.0673) versus the laboratory-reared cohorts combined (2.0000±0.0673) were significantly different (p=.0095) (Table 13: "Wild vs Laboratory"). Comparisons of least squares means of *L. nigrinus* adult females for PNW cohort (1.8438±0.0778) versus the combined data from the NC, PNW-F_1, and NC-F_1 cohorts (1.9271±0.0778) indicated no significant differences at (p=.2930) (Table 13: "Western vs Eastern").

Table 13: Cohort contrasts of *L. nirginus* live adult females. "Wild" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) and the North Carolina Wild-Caught (NC) cohorts, while "Laboratory" represents the least squares mean of the Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1) and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts. "Western" represents the least squares mean of the Pacific Northwest Wild-Caught (PNW) while "Eastern" represents the least squares mean of the combined remaining cohorts: North Carolina (NC), Pacific Northwest Laboratory-Reared F_1 Generation (PNW-F_1), and the North Carolina Laboratory-Reared F_1 Generation (NC-F_1) cohorts.

Live Adult Female Contrasts				
Label	Least Squares Mean		t Value	Pr > F
Wild vs Laboratory	Wild 1.8125±0.0673	Laboratory 2.0000±0.0673	-2.78	0.0095
Western vs Eastern	Western 1.8438 ±0.0778	Eastern 1.9271±0.0778	-1.07	0.2930

Hybridization:

The results of the genetic analyses were not clear due to possible polymerase chain reaction (PCR) stutter, which can confuse DNA profile interpretation. This indicates that there could have been inconsistencies in the number and location of peaks and alleles between individual samples. Since the test was performed to identify if there was an intermittent number of alleles between what is expected for *L. ngirinus* and what is expected for *L. rubidus*, my results were inconclusive in determining whether there were *L. nigrinus* x *L. rubidus* hybrids among the specimens I examined. However, genetic analyses of the six NC specimens in 2013

indicated possible hybridization of five of these specimens, with at least one locus in the specimen indicating a number of alleles different from what was expected according to Klein et al. (2010) for non-hybrid *L. nigrinus* (Table 14).

Table 14: Allele presence in two Pacific Northwest Wild-Caught (PNW) and six North Carolina Wild-Caught (NC) specimens for three loci, AlCA04, LaGT07, and LaGT19 and the determination whether based on present alleles, the sample was a hybrid.

Cohort	Sample Name	Locus	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Hybrid (Y/N)
PNW	1_L19_A05	LaGT19	198	190	192						Control
PNW	1_L4_A01	AlCA04	195	200	211	233	235	247	249	251	Control
PNW	1_L7_A03	LaGT07	178	229	233	235					Control
PNW	2_L19_B05	LaGT19	190	191	193						Control
PNW	2_L4_B01	AlCA04	195	200	226	227	229	231			Control
PNW	2_L7_B03	LaGT07	178		235						Control
NC	3_L19_C05	LaGT19	190	191	192						N
NC	3_L7_C03	LaGT07	178		236						N
NC	4_L19_D05	LaGT19	190	191	192						N
NC	4_L4_D01	AlCA04	97	216	218	220	222				Possible
NC	4_L7_D03	LaGT07	229	233							N
NC	5_L19_E05	LaGT19	189	190	192						N
NC	5_L4_B01	AlCA04	97	218	220	221	222				Possible
NC	5_L7_E03	LaGT07	231	232	233						Possible
NC	6_L19_F05	LaGT19	190	191	192						N
NC	6_L4_F01	AlCA04	97	200	251	253	255	257			Possible
NC	6_L7_F03	LaGT07	231	232	233						N
NC	7_L19_G05	LaGT19	190	192	193						N
NC	7_L4_G01	AlCA04	204	216	217	218					Possible
NC	7_L7_G03	LaGT07	233	234	236	237					Possible
NC	8_L19_H05	LaGT19	189	191	192						N
NC	8_L4_H01	AlCA04	97	200	241	243	245	247			Possible
NC	8_L7_H03	LaGT07	224	225	226						Possible

CHAPTER FIVE

DISCUSSION

Primary Research Question:

Do wild caught *L. nigrinus* from either the PNW or NC display significantly higher fecundity – defined here as larvae per female – than their respective F_1 generations (PNW-F_1 or NC-F_1)?

Larvae per Female:

In 2013, both wild-caught cohorts (PNW and NC) produced greater numbers of larvae per female than their laboratory-reared F_1 generations (PNW-F_1 and NC-F_1). However, due to limited replications in 2013, I could not determine whether those differences were significant. Analyses of the detailed data in 2014 supported my observations from 2013, indicating that both wild-caught cohorts produced significantly more larvae than their F_1 laboratory-reared offspring.

Hybridization:

There is a recently recognized presence of *L. nigrinus* x *L. rubidus* hybrids in the Eastern U.S. It is not known whether these hybrids represent a new species in hybrid zones or what the ultimate impact hybrids will have on biological control efforts for *A. tsugae*. However, there is evidence of hybrid fertility over successive generations (Havill et al., 2012). It is not known what impact hybridization may have on the native populations of *L. rubidus* in the Eastern U.S. Wild-caught hybrids have been shown to respond similarly

to eastern hemlock foliage infested with *A. tsugae*, as do *L. nigrinus* wild-caught in the Eastern U.S. (Arsenault, 2013). Previous collections of *Laricobius* adults from sites near Banner Elk, NC show that *L. nigrinus* x *L. rubidus* hybrids make up between 8.8 and 28.3% of the *Laricobius* population found on eastern hemlock (Arsenault, 2013).

Although the SSR (simple sequence repeat) profiles in my genetic analyses of six NC individuals were not completely clear, they resembled data from Klein et al. (2010) for *L. nigrinus*. I propose there is a chance that the samples tested, which were determined to be possible hybrids, could have been *L. nigrinus* x *L. rubidus* hybrids with *L. nigrinus* backcrossing. Such backcrossing may make it more difficult to detect hybridization, especially in my test, which only examined three loci. Despite the uncertainty in my data, considering the evidence of hybrids in area where my NC cohort was collected (Arsenault, 2013), it is likely that hybrids were present in both my NC and NC-F_1 cohorts. If hybrids have decreased larvae per female compared to non-hybridized *L. nigrinus*, this could be a cause of the lower number of larvae per female I found in in the NC versus the PNW cohorts and the NC-F_1 versus the PNW-F_1 cohorts. I recommend that future studies involving *L. nigrinus* x *L. rubidus* hybrids assess the fecundity of known hybrids compared to pure *L. nigrinus* strains to determine whether hybridization has a noticeable impact on fecundity.

In future genetic studies, I would recommend including known *L. rubidus*

specimens, which I was unable to attain. I would also use additional loci in order to determine if individuals are true hybrids. Based on suggestions by C. Saski, I would recommend that appropriate alternative methods be used to resolve the hybrid question by identifying species-specific SNP (single nucleotide polymorphisms) markers. This marker type is superior as these are sequence-based markers and resolution is at the nucleotide level (C. Saski, personal communication, July 17, 2014).

Eastern Versus Western Hemlock:

Another difference between the PNW cohort and the other three cohorts is that until PNW beetles were brought to the laboratory, they subsisted on *A. tsugae* feeding on western hemlock. The other three cohorts had fed only on *A. tsugae* feeding on eastern hemlock prior to and during my study. To see if this possibly had an effect, I compared fecundity of the PNW cohort (Western) to the three other cohorts (Eastern). *Laricobius nigrinus* collected from western hemlocks have been shown to prefer this species to eastern hemlocks as a feeding site (Wallin et al., 2011). That study also reported that laboratory-reared *L. nigrinus* fed exclusively on *A. tsugae* on eastern hemlock were generally unresponsive to odors from either type of hemlock; only 36% of the laboratory-reared beetles were in fields with plant material at the end of the experiment, compared to 84% of field-caught beetles (Wallin et al., 2011). In my study, *L. nigrinus* that had fed previously on *A. tsugae* from western hemlock produced significantly more larvae per female than those in cohorts, which had previously fed on *A. tsugae* from eastern

hemlock. This supports the findings of Wallin et al. (2011) that *A. tsugae* feeding on western hemlock may be a better food resource for *L. nigrinus* than those feeding on eastern hemlock.

I propose that this change in diet may be detrimental to the fecundity of *L. nigrinus*. I recommend further study into the impact of food, comparing laboratory, field insectary, and wild-caught cohorts of *L. nigrinus* reared exclusively on western hemlock versus laboratory, field insectary (to control for potential hybridization), and wild-caught cohorts reared exclusively on eastern hemlock.

Eggs, Live Larvae, and Total (Live plus Dead) Larvae:

The number of eggs, live larvae, and total (live plus dead) larvae were used as indicators to determine whether fecundity, based on total larvae per female was related to the greatest number of eggs, live larvae, and total larvae reared in a laboratory setting.

Eggs:

The 2013 egg data were not accurate and were therefore not used in the study. In 2014, egg data indicated that the PNW cohort should have the highest number of larvae per female based on egg counts while the NC-F_1 cohort should have the lowest overall fecundity based on egg counts. The significantly higher egg numbers in the PNW compared to the NC cohort is an indication that PNW beetles produce more eggs per female. However, as only about half of all eggs for each cohort developed to live larvae

in 2014, other analyses had to be considered in order to determine which cohort was most likely to produce the highest number of offspring in a laboratory setting.

Live Larvae and Total (Live plus Dead) Larvae:

In 2014, both the number of live larvae and total (live plus dead) larvae indicated that the wild-caught cohorts had the greatest potential to produce the highest number of offspring. Based on my results, for the purpose of mass rearing, it is irrelevant whether PNW or NC beetles are reared, as they produce similar numbers of offspring. However, it is useful to note that each year the PNW cohort had the highest number of larvae per female. My results suggest that when each cohort began with a 1:1 male:female ratio and the same number of adults, cohort fecundity could be estimated to determine which cohort will produce the greatest number of total and live offspring in a laboratory setting.

In 2013, the preliminary data for both live larvae and total larvae suggested that the PNW cohort produced the fewest total larvae. Because of the high male:female discrepancy within the PNW cohort, I concluded that the difference in the numbers of both live larvae and total larvae between 2013 and 2014 was directly related to the low number of females and not to the number of larvae each female produced.

Summary of Live Larvae and Total (Live plus Dead) Larvae 2014:

The least squares means of both live larvae (Table 4) and total larvae (live larvae plus dead larvae) (Table 6) can be used as indicators of how many larvae can potentially be

produced per week in a laboratory setting. Significantly lower least squares means of live larvae versus total larvae would indicate high larval mortality. Although the least squares means of larvae per female was used as an indicator of fecundity in this study, comparing rates of larval mortality among cohorts may indicate whether there are other variables affecting larval production in a laboratory setting.

Gender Distribution and Adult Mortality:

In 2013 at Week 0, the PNW cohort had approximately twice as many males as females; all other cohorts had approximately one male per female. The PNW cohort exhibited the greatest adult mortality, particularly of females in 2013. In 2014, the three Eastern U.S. cohorts ranked in the same relative order for live larvae and total larvae as they did in 2013 (NC>PNW-F_1>NC-F_1). In the PNW cohort, the skewed gender distribution and high level of female mortality in 2013 likely contributed to this cohort having the lowest numbers of both live and total larvae.

In 2013, the PNW cohort exhibited greater mortality than any of the other cohorts. The two key variables distinguishing the PNW cohort from the other three cohorts were: 1) they were shipped overnight across country after being collected, and 2) they had fed on *A. tsugae* on western hemlock during development. It is likely that either the stress of shipping, changes in their primary food source, or a combination of these factors were causes of the high rate of mortality. Although the PNW cohort had a higher rate of mortality compared to the other cohorts in 2014, I did not observe the skewed sex ratio in

the PNW as I had in 2013. This may suggest that the 2013 pairing of a low number of females with a high number of males led to higher female mortality in a colony. It is also worthy to note that *L. nigrinus* males consume significantly more *A. tsugae* eggs than do females (Vieira et al., 2012). It is plausible that in 2013, males outcompeted females for food leading to a continued change in sex distribution that favored males. For anyone maintaining a colony of *L. nigrinus*, I would strongly suggest determining the gender of individuals prior to setting up mating containers, and either using a 1:1 sex ratio or avoiding a ratio heavily skewed in favor of males at the beginning of a mass-rearing project.

Male and Female Loss 2014:

The relatively high mortality of both males and females observed in the PNW cohort in 2013 were not observed in this cohort in 2014. While there were some significant differences in the loss of *L. nigrinus* males and females among cohorts in 2014, and an overall cohort difference for females these losses did not reflect trends of losses by cohort in 2013. It is possible that in a mass-rearing situation, these differences would not have been significant.

Conclusion:

My study found a significant difference in larvae per females between laboratory-reared and wild-caught *L. nigrinus* for both native and naturalized populations. I concluded that laboratory rearing of *L. nigrinus* over a single generation resulted in a significant

decrease in larvae per female. While Lamb et al. (2005) suggested that a wild-caught colony can be expected to produce approximately two to four times the number of larvae that a laboratory-reared colony can be expected to produce, my results indicate that the differences may generally be less dramatic. Results from my study in 2014 (in which all cohorts began with the same number of adults and no adults were replaced) showed that the PNW wild-caught colony produced approximately 1.546 times as many live larvae as did the PNW-F_1 laboratory-reared cohort. The PNW cohort produced approximately 1.503 total larvae compared to the PNW-F_1 cohort. The NC wild-caught colony produced approximately 1.632 times the number of live larvae and 1.672 times the number of total larvae that the NC-F_1 laboratory-reared cohort produced. Therefore, according to my data you would need an initial colony of approximately 1500 to 1600 laboratory-reared PNW-F1 or NC-F1 beetles to produce as many larvae as would be expected in a colony of 1000 wild-caught PNW adults or 1000 wild-caught NC beetles respectively.

In 2014, the PNW cohort exhibited the highest measures for all fecundity parameters (eggs, live larvae, total larvae, larvae per female). For successful mass production, I would recommend beginning colonies with a 1:1 male:female ratio (or a ratio that is not heavily skewed toward males). In the absence of PNW *L. nigrinus,* PNW-F_1 *L. nigrinus* could be a viable substitute, but larval production should be expected to be significantly lower than when beginning with wild-caught PNW individuals. Although the NC cohort showed no significant difference in fecundity compared to the PNW cohort, using wild-caught NC beetles removes ovipositing *L. nigrinus* from the field where they are

functioning as biological control agents. Using NC beetles may potentially lead to propagating *L. nigrinus x L. rubidus* hybrids in the laboratory. I would not recommend the use of NC-F_1 *L. nigrinus* as this cohort produced the fewest number of larvae per female and had significantly lower least squares means of larvae per female compared to both of the wild-caught cohorts in 2014.

My results suggest that for the purpose of mass rearing adult *L. nigrinus*, colonies should begin with beetles collected in the Pacific Northwest. Those individuals should only be used to produce a single generation of larvae to be released on infested hemlock in the Eastern U.S. Because the NC cohort in my study exhibited similar larval production to the PNW cohort, use of local field-insectaries may be more efficient than laboratories for production of beetles. I strongly recommend further research into the use of field insectaries for the purpose of rearing *L. nigrinus*. I believe that continued mass rearing and release of this species should be done only with careful consideration to the consequences releases may have on existing *L. rubidus* populations and the species on which *L. rubidus* depend for sustenance.

APPENDICES

Appendix A Oviposition Charts 2013:

*R-Recovered-beetles that were originally recorded as "missing" but were later found on oviposition twigs and returned to holding jars (jars not used directly in the study)

*Notes: "X from Y" refers to how many beetles (X) were added to the jar, and from which jar they were obtained (Y). For example "4 from 14" means 4 beetles were added from Jar 14 to the jar on which the note is mentioned.

* Data for eggs, live larvae, and dead larvae was only gathered from jars 1-8; jars >8 were holding jars.

Week 0	March 6-March 13				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	26	3	1	
2	30	28	2	0	
3	30	25	3	2	
4	30	27	1	2	
5	30	25	5	0	
6	30	27	3	0	
7	30	27	3	0	
8	30	25	2	3	
9	30	27	3	0	
10	30	29	1	0	
11	30	26	2	2	
12	30	25	4	1	
13	30	28	1	1	
14	10	7	3	0	
Recovered		8 (March 15)	3 (March 15)		
Total	400	360	39	12 (1 after R)	

Week 1	March 13-March 20				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	27	2	1	4 from 14
2	30	28	1	1	2 from 14
3	30	26	3	1	5 from 13
4	30	28	2	0	3 from 13
5	30	26	3	1	5 from 13
6	33	29	1	3	6 from 13
7	30	27	2	1	3 from 13
8	30	26	4	0	5 from 13
9	27	24	3	0	
10	30	30	0	0	1 from R
11	30	29	1	0	2 from R + 2 from 13
12	30	28	1	1	5 from R
Recovered		2 (March 20)			
Total	360	330	23	9 (7 after R)	

Week 2	March 20-March 27				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	25	3	2	3 from 12
2	30	28	2	0	2 from 12
3	30	27	3	0	4 from 12
4	30	28	2	0	2 from 12
5	30	23	7	0	4 from 12
6	30	29	1	0	1 from 12
7	30	29	1	0	3 from 12
8	30	29	1	0	4 from 12
9	30	28	0	2	5 from 12 + 1 from R
10	30	27	2	1	
11	30	26	3	1	1 from R
Recovered		1 (March 28)			
Total	330	300	25	6 (5 after R)	

Week 3	March 27-April 3				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	1	0	5 from 11
2	30	26	4	0	2 from 11
3	30	29	0	1	3 from 11
4	30	25	4	1	2 from 11
5	30	30	0	0	7 from 11
6	30	27	3	0	1 from 11
7	30	30	0	0	1 from 11
8	30	26	4	0	1 from 11
9	30	26	2	2	2 from 11
10	30	28	2	0	2 from 11 + 1 from R
Recovered		2 (April 3 & 4)			
Total	300	278	20	4 (2 after R)	

Week 4	April 3-April 10				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	28	1	1	1 from 10
2	30	29	1	0	4 from 10
3	31	28	3	0	2 from 10
4	30	28	2	0	5 from 10
5	30	25	4	1	
6	30	27	3	0	3 from 10
7	30	25	4	1	
8	30	26	2	2	4 from 10
9	37	31	5	1	9 from 10 + 2 from R
Recovered		1 (April 11)			
Total	278	248	25	6 (5 after R)	

Week 5	April 10- April 17				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	26	3	1	2 from 9
2	30	27	2	1	1 from 9
3	30	25	3	2	2 from 9
4	30	27	1	2	2 from 9
5	30	28	2	0	5 from 9
6	30	27	2	1	3 from 9
7	30	28	1	1	5 from 9
8	30	27	2	1	4 from 9
9	8	7	0	1	1 from R
Recovered		7 (April 18 & 19)	1 (April 18)		
Total	248	229	17	10 (2 after R)	

Week 6	April 17- April 24				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	1	0	4 from 9
2	30	29	1	0	3 from 9
3	30	29	1	0	5 from 8
4	30	28	1	1	3 from 8
5	30	27	2	1	2 from 8
6	30	28	2	0	3 from 8
7	30	26	1	3	2 from 8
8	19	17	1	1	7 from R
Recovered		4 (April 25)			
Total	229	217	10	6 (2 after R)	

Week 7	April 24-May 1				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	1 from 8
2	30	29	0	1	1 from 8
3	30	28	0	2	1 from 8
4	30	27	2	1	2 from 8
5	30	25	3	2	3 from 8
6	30	28	2	0	2 from 8
7	30	29	0	1	4 from 8
8	7	7	0	0	4 from R
Recovered		4 (May 2 & 3)			
Total	217	206	7	8 (4 after R)	

Week 8	May 1-May 8				
PNW					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	28	0	2	1 from 8
2	30	28	1	1	1 from 8
3	30	28	1	1	2 from 8
4	30	26	3	1	3 from 8
5	30	28	1	1	5 from 7
6	30	27	2	1	2 from 7
7	26	23	3	0	4 from R
Recovered		3 (May 8 & 9)			
Total	206	191	11	7	

Week 0	March 4-March 11				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	30	0	0	
2	30	30	0	0	
3	30	25	4	1	
4	30	30	0	0	
5	30	29	0	1	
6	30	28	0	2	
7	30	30	0	0	
8	30	26	3	1	
9	30	29	0	1	
10	30	28	1	1	
11	30	26	2	2	
12	37	33	0	4	
Recovered		7 (March 14)			
Total	367	351	10	13 (6 after R)	

Week 1	March 11-March 18				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	
2	30	28	2	0	
3	25	23	1	1	
4	30	30	0	0	
5	29	28	0	1	
6	28	22	2	4	
7	30	27	2	1	
8	26	23	1	2	
9	30	30	0	0	1 from R
10	30	27	1	2	2 from R
11	30	27	2	1	4 from R
12	33	33	0	0	
Recovered		3 (March 18)			
Total	351	330	11	13 (10 after R)	

Week 2	March 18-March 25				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	26	0	4	1 from 11
2	30	29	1	0	2 from 11
3	30	29	0	1	7 from 11
4	30	26	0	4	
5	30	27	2	1	2 from 11
6	30	26	1	3	8 from 11
7	30	29	1	0	3 from 11
8	30	25	3	2	4 from 11 + 3 from 12
9	30	26	2	2	
10	30	27	3	0	3 from R
11	30	28	2	0	Formerly jar "12"
Recovered		4			
Total	330	302	15	17 (13 after R)	

Week 3	March 25-April 1				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	25	2	3	4 from 11
2	30	26	3	1	1 from 11
3	30	28	0	2	1 from 11
4	30	27	3	0	4 from 11
5	30	28	2	0	3 from 11
6	30	31	0	0	4 from 11 (1 Extra)
7	30	29	0	1	1 from 11
8	30	30	0	0	5 from 11
9	30	30	0	0	4 from 11
10	32	28	0	4	1/4 f 11/R
Recovered		2 (April 2)			
Total	302	284	10	11 (9 after R)	

Week 4	April 1- April 8				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	5 from 10
2	30	28	2	0	4 from 10
3	30	29	0	1	2 from 10
4	30	25	4	1	3 from 10
5	30	28	2	0	2 from 10
6	30	29	1	0	(1 Red)
7	30	29	1	0	1 from 6
8	30	25	3	2	2 from 10
9	30	29	0	1	
10	12 (14?)	10	0	4	2 from R (4/2)?
Recovered					
Total	284	261	13	10	

Week 5	April 8- April 15				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	1 from 10
2	30	28	1	1	2 from 10
3	30	27	2	1	1 from 10
4	30	26	1	3	5 from 10
5	30	30	0	0	2 from 10
6	30	30	0	0	1 from 9
7	30	29	1	0	1 from 9
8	30	29	1	0	5 from 9
9	21	21	0	0	
Recovered		3 (April 16)			
Total	261	252	6	6 (3 after R)	

Week 6	April 15-April 22				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	1	0	1 from 9
2	30	30	0	0	2 from 9
3	30	28	2	0	3 from 9
4	30	29	0	1	4 from 9
5	30	28	2	0	
6	30	29	1 (artificially)	0	(1 Red-died 4/23-Replaced by black)
7	30	29	0	1	1 from 9
8	30	28	1	1	1 from 9
9	12	10	1	1	3 from R
Recovered		2 (April 23)			
Total	252	242	8	4 (2 after R)	

Week 7	April 22-April 29				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	1 from 9
2	30	29	0	1	
3	30	27	2	1	2 from 9
4	30	29	1	0	1 from 9
5	30	29	1	0	2 from 9
6	30	28	0	2	1 from 9
7	30	28	0	2	1 from 9 (1 "dead" revived and moved to 8-count as Missing)
8	30	29	0	1	2 from 9
9	2	2	0	0	2 from R
Recovered		3 (April 29 & 30)			
Total	242	233	4	8 (5 after R)	

Week 8	April 29- May 6				
PNW-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	26	2	2	1 from 9
2	30	27	2	1	1 from 9
3	30	29	0	1	3 from 8
4	30	29	1	0	1 from 8
5	30	27	2	1	2 from 8
6	30	28	1	1	2 from 8
7	30	27	2	1	2 from 8
8	23	22	0	1	1 from 7 + 3 from 3
Recovered		6 (May 6)			
Total	233	221	10	8 (2 after R)	

Week 0	March 7- March 14				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	30	0	0	
2	30	28	1	1	
3	30	27	2	1	
4	30	30	0	0	
5	30	29	0	1	
6	30	30	0	0	
7	30	28	1	1	
8	30	28	0	2	
9	30	28	0	2	
10	30	28	0	2	
11	30	28	0	2	
12	12	11	0	1	
Recovered		10			
Total	342	335	4	13 (3 after R)	

Week 1	March 14-March 21				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	30	0	0	
2	30	30	0	0	
3	30	30	0	0	
4	30	25	3	2	
5	30	29	1	0	
6	30	30	0	0	
7	30	28	1	1	
8	30	29	0	1	
9	30	29	1	0	
10	35	35	0	0	
11	30	30	0	0	
Recovered		3			
Total	335	328	6	4 (1 after R)	

Week 2	March 21-March 28				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	
2	30	30	0	0	
3	30	26	3	1	
4	30	28	1	1	5 from 10
5	30	28	0	2	1 from 11
6	30	29	0	1	
7	30	28	0	2	2 from 11
8	30	29	0	1	1 from 11
9	31	31	0	0	1 from 11 + 1 from R
10	30	29	0	1	
11	27	25	0	2	2 from R
Recovered		5 (March 29)			
Total	328	317	4	12 (7 after R)	

Week 3	March 28-April 4				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	30	0	0	1 from 11
2	30	30	0	0	
3	30	29	0	1	4 from 11
4	30	30	0	0	2 from 11
5	30	29	0	1	2 from 11
6	30	30	0	0	1 from 11
7	30	30	0	0	2 from 11
8	30	29	0	1	1 from 11
9	38	36	1	1	5 from 11 + 2 from R
10	39	39	0	0	7/3 from 11/R
Recovered		3 (April 5)			
Total	317	315	1	4 (1 after R)	

Week 4	April 4-April 11				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	28	0	2	
2	30	29	1	0	
3	30	30	0	0	1 from 10
4	30	29	0	1	(1 Red)
5	30	29	0	1	1 from 10
6	30	30	0	0	
7	30	30	0	0	(1 Red)
8	30	28	0	2	1 from 10
9	37	37	0	0	1 from R
10	38	35	1	2	2 from R
Recovered		6 (April 12)			
Total	315	311	2	8 (2 after R)	

Week 5	April 11- April 18				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	1	0	2 from 10
2	30	29	0	1	1 from 10
3	30	29	0	1	
4	30	29	0	1	1 from 10 (1 Red)
5	30	30	0	0	1 from 10
6	30	30	0	0	
7	30	29	0	1	
8	30	30	0	0	2 from 9
9	35	33	1	1	
10	36	34	1	1	6 from R
Recovered		6 (April 19,20, 24)			
Total	311	308	3	6 (0 after R)	

Week 6	April 18- April 25				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	1	0	1 from 10
2	30	30	0	0	1 from 10
3	30	28	1	1	1 from 10
4	30	29	0	1	1 from 10 (1 Red)
5	30	29	0	1	(1 Red)
6	30	27	1	2	
7	30	30	0	0	1 from 10 (1 Red)
8	30	28	2	0	
9	34	32	0	2	1 from R
10	34	34	0	0	5 from R
Recovered		3 (April 26 & 27)	1 (April 27)		
Total	308	299	6	7 (3 after R)	

Week 7	April 25-May 2				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	30	0	0	1 from 10
2	30	29	0	1	
3	30	28	0	2	2 from 10
4	30	29	0	1	1 from 10
5	30	29	0	1	1 from 10
6	30	29	0	1	3 from 10
7	30	25	1	4	
8	30	28	0	2	2 from 10
9	32	29	1	2	
10	27	25	1	1	3 from R
Recovered		9 (May 3,4, 5)	1 (May 3)		
Total	299	290	4	15 (5 after R)	

Week 8	May 2-May 9				
NC					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	28	0	2	
2	30	27	0	3	1 from 10
3	30	29	1	0	2 from 10
4	30	30	0	0	1 from 10
5	30	29	0	1	1 from 10
6	30	25	3	2	1 from 10
7	30	28	0	2	5 from 10
8	30	29	1	0	2 from 10
9	35	31	3	1	6 from R
10	15	14	0	1	3 from R
Recovered		6 (May 9 &10)			
Total	290	276	8	12 (6 after R)	

Week 0	March 5-March 12				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	27	0	3	
2	30	27	2	1	
3	30	29	0	1	
4	30	29	0	1	
5	30	27	2	1	
6	30	29	0	1	
7	30	27	2	1	
8	30	29	0	1	
9	30	28	0	2	
10	30	26	2	2	
11	16	14	1	1	
Recovered		8 (March 12 & 14)			
Total	316	300	9	15 (7 after R)	

Week 1	March 12-March 19				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	3 from 11
2	30	29	1	0	3 from 11
3	30	28	0	2	1 from 11
4	30	28	2	0	1 from 11
5	30	28	2	0	3 from 11
6	30	28	2	0	1 from 11
7	30	27	3	0	1 from 10 + 2 from 11
8	30	29	0	1	1 from 10
9	30	28	1	1	2 from R
10	30	28	1	1	5 from R
Recovered					
Total	300	282	12	6	

Week 2	March 19-March 26				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	1 from 10
2	30	29	0	1	1 from 10
3	30	29	1	0	2 from 10
4	30	28	1	1	2 from 10
5	30	29	0	1	2 from 10
6	30	29	0	1	2 from 10
7	30	28	1	1	3 from 10
8	30	30	0	0	1 from 10
9	28	25	2	1	
10	14	12	1	1	
Recovered		4			
Total	282	272	6	8 (4 after R)	

Week 3	March 26-April 2				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	29	0	1	1 from 10
2	30	30	0	0	1 from 10
3	30	29	0	1	1 from 10
4	30	29	0	1	2 from 10
5	30	30	0	0	1 from 10
6	30	27	2	1	1 from 10
7	30	29	0	1	2 from 10
8	30	28	2	0	
9	32	31	0	1	3/4 from 10/R
Recovered		3 (April 3)			
Total	272	265	4	6 (3 after R)	

Week 4	April 2-April 9				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	28	1	1	1 from 9
2	30	29	0	1	
3	30	25	4	1	1 from 9
4	30	30	0	0	1 from 9
5	30	27	3	0	
6	30	30	0	0	3 from 9
7	30	30	0	0	1 from 9
8	30	30	0	0	2 from 9
9	25	24	1	0	3 from R
Recovered		2 (April 9 & April 10)			
Total	265	255	9	3 (1 after R)	

Week 5	April 9-April 16				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	27	0	3	2 from 9
2	30	28	1	1	1 from 9
3	30	22	1	7	5 from 9
4	30	25	1	4	
5	30	28	1	1	3 from 9
6	30	27	0	3	
7	30	26	2	2	
8	30	28	0	2	
9	15	12	0	3	2 from R
Recovered		15 (April 16)	1		
Total	255	238	6	26 (10 after R)	

Week 6	April 16-April 23				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	29	29	0	0	2 from 9
2	30	28	1	1	2 from 9
3	30	30	0	0	8 from 9
4	30	29	0	1	5 from 8
5	30	28	2	0	2 from 8
6	30	28	2	0	3 from 8
7	30	24	1	5	4 from 8
8	29	26	2	1	15 from R (1 Red)
Recovered		3 (April 24)			
Total	238	225	8	8 (5 after R)	

Week 7	4/23-4/30				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	28	0	2	1 from 8
2	30	29	1	0	2 from 8 (1 Red)
3	30	28	1	1	
4	30	29	0	1	1 from 8
5	30	28	1	1	2 from 8
6	30	30	0	0	2 from 8
7	30	29	1	0	6 from 8
8	15	15	0	0	3 from R (1 Red)
Recovered		6 (April 30 & May 1)			
Total	225	222	4	5 (-1 after R?)	

Week 8	April 30- May 7				
NC-F1					
Jars	Beetles	Living	Dead	Missing	Notes
1	30	26	2	2	2 from 8
2	30	28	0	2	1 from 8
3	30	27	1	2	2 from 8
4	30	29	0	1	1 from 8
5	30	28	0	2	2 from 8
6	30	29	0	1	
7	30	29	0	1	1 from 8
8	12	12	0	0	6 from R
Recovered		6 (May 7 & 8)			
Total	222	214	3	11 (5 after R)	

Appendix B Gender Charts 2013:

*Ln- *L. nigrinus* *M-Males *F-Females *Final Week=end of week 8

* Data for eggs, live larvae, and dead larvae was only gathered from jars 1-8; jars >8 were holding jars.

Week 1 Date: June 11 Cohort: PNW

Ln Jar	M	F
1	0	2
2	0	1
3	2	1
4	1	1
5	1	2
6	0	1
7	0	2
8	0	4
9	1	2
10	0	0
11	0	1
12	1	0

Week 2 Date: June 14 Cohort: PNW

Ln Jar	M	F
1	0	2
2	2	1
3	0	3
4	1	1
5	3	4
6	1	0
7	0	1
8	1	0
9	0	0
10	1	1
11	0	3

Week 3 Date: June 17 Cohort: PNW

Ln Jar	M	F
1	1	0
2	0	4
3	0	0
4	1	3
5	0	0
6	0	3
7	0	0
8	0	4
9	1	1
10	1	1

Week 4 Date: June 18 Cohort: PNW

Ln Jar	M	F
1	0	1
2	1	0
3	0	3
4	1	1
5	1	3
6	0	3
7	1	3
8	0	2
9	2	2
10	0	0

Week 5 Date: June 19 Cohort: PNW

Ln Jar	M	F
1	1	1
2	0	2
3	1	2
4	1	0
5	2	0
6	0	2
7	1	0
8	0	1
9	0	0
10	0	1

Week 6 Date: June 24 Cohort: PNW

Ln Jar	M	F
1	1	0
2	0	1
3	1	0
4	1	0
5	2	0
6	0	2
7	0	1
8	0	1
9	0	0
10	0	0

Week 7 Date: June 24 Cohort: PNW

Ln Jar	M	F
1	0	0
2	0	0
3	0	0
4	1	1
5	1	2
6	2	0
7	0	0
8	0	0
9	0	0
10	0	0

Week 8 Date: June 25 Cohort: PNW

Ln Jar	M	F
1	0	0
2	0	1
3	0	1
4	2	1
5	1	0
6	0	2
7	2	1
8	0	0
9	0	0
10	0	0

Final Week Date: July 1 Cohort: PNW 2013

Ln Jar	M	F
1	26	3
2	26	2
3	19	6
4	25	1
5	23	1
6	24	3
7	21	2
R	3	0

Week 1 Date: June 11 Cohort: PNW-F_1

Ln Jar	M	F
1	0	0
2	0	2
3	1	0
4	0	0
5	0	0
6	1	1
7	0	2
8	0	1
9	0	0
10	0	0

Week 2 Date: June 13 Cohort: PNW-F_1

Ln Jar	M	F
1	0	0
2	0	1
3	0	3
4	0	0
5	0	2
6	0	1
7	0	1
8	0	0
9	0	2
10	1	2
11	0	0

Week 3 Date: June 14 Cohort: PNW-F_1

Ln Jar	M	F
1	0	2
2	1	2
3	0	0
4	0	3
5	0	2
6	0	0
7	0	0
8	0	2
9	0	0
10	0	0

Week 4 Date: June 18 Cohort: PNW-F_1

Ln Jar	M	F
1	0	0
2	1	1
3	1	0
4	2	2
5	1	1
6	0	1
7	0	1
8	0	3
9	0	0
10	0	0

Week 5 Date: June 19 Cohort: PNW-F_1

Ln Jar	M	F
1	0	0
2	0	1
3	0	2
4	1	0
5	0	0
6	0	0
7	0	1
8	1	0
9	0	0
10	0	0

Week 6 Date: June 24 Cohort: PNW-F_1

Ln Jar	M	F
1	1	0
2	0	0
3	0	2
4	0	0
5	1	1
6	0	1
7	0	0
8	0	1
9	0	1
10	0	0

Week 7 Date: June 24 Cohort: PNW-F_1

Ln Jar	M	F
1	0	0
2	0	0
3	1	1
4	0	1
5	0	2
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Week 8 Date: June 25 Cohort: PNW-F_1

Ln Jar	M	F
1	1	1
2	0	2
3	0	0
4	1	0
5	1	1
6	1	0
7	0	2
8	0	0
9	0	0

Final Week Date: June 30 Cohort: PNW-F_1

Ln Jar	M	F
1	18	8
2	13	14
3	14	15
4	16	13
5	15	12
6	17	9
7	16	10
8	18	4
R	6	1

Week 1 Date June 11 Cohort: NC

Ln Jar	M	F
1	0	0
2	0	0
3	0	0
4	2	1
5	0	1
6	0	0
7	0	1
8	0	0
9	1	0
10	0	0

Week 2 Date: June 14 Cohort: NC

Ln Jar	M	F
1	0	0
2	0	0
3	2	1
4	0	1
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Week 3 Date: June 17 Cohort: NC

Ln Jar	M	F
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	1	0
10	0	0

Week 4 Date: June 18 Cohort: NC

Ln Jar	M	F
1	0	0
2	0	1
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	1

Week 5 Date: June 19 Cohort: NC

Ln Jar	M	F
1	0	1
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	1
10	0	1

Week 6 Date: June 24 Cohort: NC

Ln Jar	M	F
1	1	0
2	0	0
3	0	1
4	0	0
5	0	0
6	0	1
7	0	0
8	1	1
9	0	0
10	0	0
R	1	0

Week 7 Date: June 24 Cohort: NC

Ln Jar	M	F
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	1	0
8	0	0
9	0	1
10	0	0
R	0	1

Week 8 Date: June 25 Cohort: NC

Ln Jar	M	F
1	0	0
2	0	0
3	0	1
4	0	0
5	0	0
6	2	1
7	0	0
8	0	1
9	2	1
10	0	0

Final Week Date: July 2 Cohort: NC 2013

Ln Jar	M	F
1	13	15
2	15	12
3	15	14
4	16	11
5	14	15
6	13	12
7	15	13
8	15	14
9	15	13
10	9	5
R	4	2

Week 1 Date: June 11 Cohort: NC-F_1

Ln Jar	M	F
1	0	0
2	0	1
3	0	0
4	2	0
5	1	1
6	0	1
7	2	1
8	0	0
9	0	0
10	0	0

Week 2 Date: June 13 Cohort: NC-F_1

Ln Jar	M	F
1	0	0
2	0	0
3	1	0
4	1	0
5	0	0
6	0	0
7	0	1
8	0	0
9	1	1
10	0	1

Week 3 Date: June 17 Cohort: NC-F_1

Ln Jar	M	F
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	2
7	0	0
8	0	2
9	0	0
10	0	0

Week 4 Date: June 18 Cohort: NC-F_1

Ln Jar	M	F
1	0	1
2	0	0
3	0	4
4	0	0
5	1	2
6	0	0
7	0	0
8	0	0
9	1	0
10	0	0

Week 5 Date: June 19 Cohort: NC-F_1

Ln Jar	M	F
1	0	0
2	1	0
3	0	1
4	0	1
5	1	0
6	0	0
7	0	2
8	0	0
9	0	0
10	0	1

Week 6 Date: June 24 Cohort: NC-F_1

Ln Jar	M	F
1	0	0
2	0	1
3	0	0
4	0	0
5	1	1
6	1	1
7	1	0
8	1	0
9	0	0
10	0	0

Week 7 Date: June 24 Cohort: NC-F_1

Ln Jar	M	F
1	0	0
2	0	1
3	1	0
4	0	0
5	1	0
6	0	0
7	0	1
8	0	0
9	0	0
10	0	0

Week 8 Date: June 25 Cohort: NC-F_1

Ln Jar	M	F
1	1	1
2	0	0
3	1	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Final Week Date: July 1 Cohort: NC-F_1

Ln Jar	M	F
1	15	11
2	18	10
3	15	12
4	14	15
5	16	12
6	19	10
7	14	15
8	5	7
R	4	2

Appendix C Larval Data 2013:

*Live Larvae: Edited Live Larvae to exclude data prior to 20 days after set-up of larval tents. Reason of this is explained in the Methods and Materials.

	PNW	PNW-F1	NC	NC-F1
Week 1	350	1199	761	638
Week 2	446	481	748	755
Week 3	608	656	1163	425
Week 4	272	384	701	257
Week 5	231	200	374	74
Week 6	143	396	180	159
Week 7	97	322	130	217
Week 8	83	146	38	113
Overall	2230	3784	4095	2638

Appendix D Cumulative Data with Larvae per Female Calculation 2013:

*Edited to exclude data prior to 20 days after set-up.

Pacific Northwest 2013			
Total Larvae	Adults	Females	Twigs
366	240	92/274	81/81
461	240	78/256	80/80
618	240	66/236	81/81
277	240	52/220	80/80
259	240	36/200	81/81
149	210	28/186	71/71
76	210	24/177	51/70
65	180	21/170	51/66
2271			

PNW 2013 Week	% Females	Estimated Females	% Twigs	Estimated Total Larvae	Estimated Total Larvae per Estimated Total Females	Total Larvae per Estimated Females
1	33.5766	81	100	366	366/81=	4.5
2	30.4687	74	100	461	461/74=	6.2
3	27.966101	68	100	618	618/68=	9.1
4	23.63636	57	100	277	277/57=	4.9
5	18	44	100	259	259/44=	5.9
6	15.05376	32	100	149	149/32=	4.7
7	13.559322	29	72.8571	104	104/29=	3.6
8	12.352941	23	77.2727	84	65/23=	3.7

Pacific Northwest-F_1 2012			
Total Larvae	Adults	Females	Twigs
1210	240	135/267	80/80
503	240	129/259	80/80
692	240	121/251	80/80
434	240	110/239	80/80
219	240	101/225	80/80
200	240	97/219	39/80
185	240	92/212	45/81
115	210	88/207	60/77
3558			

Week	% Females	Estimated Females	% Twigs	Estimated Total Larvae	Estimated Total Larvae per Estimated Total Females	Total Larvae per Estimated Females
1	50.5618	122	100	1210	1210/122=	9.9
2	49.8069	120	100	503	513/120=	4.3
3	48.2071	116	100	692	735/116=	6.3
4	46.0251	111	100	434	441/111=	3.97
5	44.8889	108	100	219	268/108=	2.5
6	44.29224	107	48.75	410	410/107=	3.8
7	43.39623	105	55.56	333	333/105=	3.2
8	42.51208	90	77.92	148	148/90=	1.6

North Carolina 2013			
Total Larvae	Adults	Females	Twigs
782	240	122/251	80/80
754	240	119/246	80/80
1175	240	117/242	80/80
720	240	117/242	81/81
386	240	116/241	62/80
193	240	115/240	55/80
136	240	112/235	34/80
39	240	111/233	34/88
4185			

NC 2013 Week	% Females	Estimated Females	% Twigs	Estimated Total Larvae	Estimated Total Larvae per Estimated Total Females	Total Larvae per Estimated Females
1	48.6055777	117	100	782	782/117=	6.68
2	48.3739837	117	100	754	782/117=	6.68
3	48.3471074	117	100	1175	1193/117=	10.197
4	48.3471074	117	100	720	768/117=	6.56
5	48.1327801	116	77.5	498	498/116=	4.29
6	47.9166667	115	68.75	281	281/115=	2.44
7	47.6595745	115	42.5	320	320/115=	2.78
8	47.639485	115	38.63636	101	101/115=	0.88

North Carolina-F_1 2012			
Total Larvae	Adults	Females	Twigs
712	240	113/246	80/80
722	240	109/236	74/80
458	240	108/233	80/80
268	240	104/229	80/80
75	240	97/221	80/80
144	210	93/215	56/70
122	210	90/209	39/70
83	210	88/205	56/77
2584			

Week	% Females	Estimated Females	% Twigs	Estimated Total Larvae	Estimated Total Larvae per Estimated Total Females	Total Larvae per Estimated Females
1	45.93495	111	100	712	712/111=	6.4
2	46.18644	111	92.5	781	781/111=	7
3	46.35193	112	100	458	509/112=	4.5
4	45.41484	109	100	268	290/109=	2.7
5	43.91403	106	100	75	77/106=	0.73
6	43.25581	91	80	180	180/91=	1.98
7	43.0622	91	55.7	219	219/91=	2.4
8	42.92683	91	72.7	114	114/91=	0.797

Appendix E Emergence of Adults from 2013 Cohorts:

Container #	Cohort	Generation	# Larva in	# Live adults
			Total:	Total live:
C49	PNW-2013	F1	20	10
B101	PNW-2013	F1	22	19
D126	PNW-2013	F1	50	49
D336	PNW-2013	F1	19	11
D69	PNW-2013	F1	20	15
D20	PNW-2013	F1	50	46
C39	PNW-2013	F1	50	44
D326	PNW-2013	F1	50	30
D67	PNW-2013	F1	45	37
D17	PNW-2013	F1	50	33
D102	PNW-2013	F1	25	18
C45	PNW-2013	F1	49	16
B106	PNW-2013	F1	50	43
C18	PNW-2013	F1	50	22
D199	PNW-2013	F1	50	40
E33	PNW-2013	F1	50	36
E008	PNW-2013	F1	50	40
E003	PNW-2013	F1	31	15
E18	PNW-2013	F1	50	43
E32	PNW-2013	F1	6	3
E007	PNW-2013	F1	50	36
B31	PNW-2013	F1	50	40
B35	PNW-2013	F1	50	31
B40	PNW-2013	F1	50	29
D328	PNW-2013	F1	19	11
D49	PNW-2013	F1	50	21
B15	PNW-2013	F1	19	0
C88	PNW-2013	F1	50	44
E16	PNW-2013	F1	1	1
D37	PNW-2013	F1	50	38
B78	PNW-2013	F1	50	42
B6	PNW-2013	F1	11	7
D104	PNW-2013	F1	50	13
C44	PNW-2013	F1	50	47
C79	PNW-2013	F1	50	44
C86	PNW-2013	F1	50	30
C37	PNW-2013	F1	50	42

B119	PNW-2013	F1	9	1
D93	PNW-2013	F1	50	41
B90	PNW-2013	F1	50	42
D330	PNW-2013	F1	50	35
C92	PNW-2013	F1	50	44
C20	PNW-2013	F1	50	31
D47	PNW-2013	F1	12	7
C32	PNW-2013	F1	50	36
C6	PNW-2013	F1	50	32
C28	PNW-2013	F1	10	0
B48	PNW-2013	F1	50	36
D156	PNW-2013	F1	5	2
B42	PNW-2013	F1	10	8
E25	PNW-2013	F1	50	36
B58	PNW-2013	F1	3	1
D198	PNW-2013	F1	50	34
C5	PNW-2013	F1	50	44
D190	PNW-2013	F1	50	33
B84	PNW-2013	F1	23	17
D158	PNW-2013	F1	50	35
D100	PNW-2013	F1	50	39
B115	PNW-2013	F1	25	18
D27	PNW-2013	F1	2	1
B67	PNW-2013	F1	18	14
D125	PNW-2013	F1	16	10
C58	PNW-2013	F1	11	5
B99	PNW-2013	F1	45	39
D116	PNW-2013	F1	3	1
D85	PNW-2013	F1	1	1

Container #	Cohort	Generation	# Larva in	# Live adults
			Total:	Total live:
D58	PNW-F1-2012	F2	22	14
D168	PNW-F1-2012	F2	30	18
B28	PNW-F1-2012	F2	50	47
D174	PNW-F1-2012	F2	39	28
D26	PNW-F1-2012	F2	50	45
C1	PNW-F1-2012	F2	5	4
C96	PNW-F1-2012	F2	50	43
E14	PNW-F1-2012	F2	50	27
C110	PNW-F1-2012	F2	50	45
C109	PNW-F1-2012	F2	50	48
D23	PNW-F1-2012	F2	50	47
B75	PNW-F1-2012	F2	50	47
D21	PNW-F1-2012	F2	50	46
D92	PNW-F1-2012	F2	43	36
D173	PNW-F1-2012	F2	50	44
B43	PNW-F1-2012	F2	50	47
C14	PNW-F1-2012	F2	50	41
H2	PNW-F1-2012	F2	50	41
D119	PNW-F1-2012	F2	50	48
D122	PNW-F1-2012	F2	50	47
D165	PNW-F1-2012	F2	50	36
D82	PNW-F1-2012	F2	50	40
D68	PNW-F1-2012	F2	50	47
D157	PNW-F1-2012	F2	50	44
D52	PNW-F1-2012	F2	50	29
D30	PNW-F1-2012	F2	50	29
D303	PNW-F1-2012	F2	50	40
D142	PNW-F1-2012	F2	50	41
D143	PNW-F1-2012	F2	50	31
D39	PNW-F1-2012	F2	34	23
D302	PNW-F1-2012	F2	50	38
D66	PNW-F1-2012	F2	50	25
B107	PNW-F1-2012	F2	50	26
E30	PNW-F1-2012	F2	50	28
E001	PNW-F1-2012	F2	50	42
E31	PNW-F1-2012	F2	50	27
D003	PNW-F1-2012	F2	50	36
D313	PNW-F1-2012	F2	34	18
D118	PNW-F1-2012	F2	10	4

D40	PNW-F1-2012	F2	50	33
B14	PNW-F1-2012	F2	33	15
D113	PNW-F1-2012	F2	50	44
B51	PNW-F1-2012	F2	12	9
D333	PNW-F1-2012	F2	50	34
C94	PNW-F1-2012	F2	50	38
B8	PNW-F1-2012	F2	50	34
B24	PNW-F1-2012	F2	49	20
B82	PNW-F1-2012	F2	50	39
C72	PNW-F1-2012	F2	50	29
B105	PNW-F1-2012	F2	29	23
C85	PNW-F1-2012	F2	50	31
D14	PNW-F1-2012	F2	50	32
C75	PNW-F1-2012	F2	1	1
E24	PNW-F1-2012	F2	50	31
E28	PNW-F1-2012	F2	50	23
D108	PNW-F1-2012	F2	50	21
C40	PNW-F1-2012	F2	11	7
D321	PNW-F1-2012	F2	50	46
D84	PNW-F1-2012	F2	24	14
D43	PNW-F1-2012	F2	25	13
D312	PNW-F1-2012	F2	50	42
B110	PNW-F1-2012	F2	4	0
D175	PNW-F1-2012	F2	50	44
D315	PNW-F1-2012	F2	50	46
B17	PNW-F1-2012	F2	50	44
B12	PNW-F1-2012	F2	50	30
D324	PNW-F1-2012	F2	50	36
B113	PNW-F1-2012	F2	12	11
E004	PNW-F1-2012	F2	50	9
C10	PNW-F1-2012	F2	50	10
D310	PNW-F1-2012	F2	50	3
B27	PNW-F1-2012	F2	39	14
D61	PNW-F1-2012	F2	50	26
B80	PNW-F1-2012	F2	18	9
D31	PNW-F1-2012	F2	4	4
B89	PNW-F1-2012	F2	50	29
D56	PNW-F1-2012	F2	4	2
D331	PNW-F1-2012	F2	50	35
B74	PNW-F1-2012	F2	1	1
B111	PNW-F1-2012	F2	50	33
B37	PNW-F1-2012	F2	19	11

D134	PNW-F1-2012	F2	50	24
B76	PNW-F1-2012	F2	50	41
D180	PNW-F1-2012	F2	50	12
B114	PNW-F1-2012	F2	8	2
C91	PNW-F1-2012	F2	50	38
D163	PNW-F1-2012	F2	25	10
C99	PNW-F1-2012	F2	12	8
D114	PNW-F1-2012	F2	1	0

Container #	Cohort	Generation	# Larva in	# Live adults
			Total:	Total live:
C102	NC-2013	F1	25	13
E29	NC-2013	F1	25	13
D325	NC-2013	F1	15	13
D151	NC-2013	F1	50	42
D154A	NC-2013	F1	13	7
D153A	NC-2013	F1	6	6
C41	NC-2013	F1	50	43
D004	NC-2013	F1	50	43
D306	NC-2013	F1	50	44
D51	NC-2013	F1	50	40
D186	NC-2013	F1	50	45
C33	NC-2013	F1	50	37
B102	NC-2013	F1	50	48
D140	NC-2013	F1	50	34
D326	NC-2013	F1	50	50
B70	NC-2013	F1	50	42
D88	NC-2013	F1	50	40
D34	NC-2013	F1	16	13
B100	NC-2013	F1	17	13
D178	NC-2013	F1	50	39
C78	NC-2013	F1	50	18
E15	NC-2013	F1	50	38
D203	NC-2013	F1	7	4
E006	NC-2013	F1	36	27
B71	NC-2013	F1	50	42
B18	NC-2013	F1	50	39
D194	NC-2013	F1	19	10
B88	NC-2013	F1	50	38
D86	NC-2013	F1	50	34
B66	NC-2013	F1	50	30
B46	NC-2013	F1	50	38
D300	NC-2013	F1	50	41
D161	NC-2013	F1	50	36
D97	NC-2013	F1	17	11
D120	NC-2013	F1	50	33
D35	NC-2013	F1	50	30
B23	NC-2013	F1	50	34
D147	NC-2013	F1	39	3
C11	NC-2013	F1	50	25

D139	NC-2013	F1	50	28
E22	NC-2013	F1	45	21
D150	NC-2013	F1	50	42
D19	NC-2013	F1	20	16
D96	NC-2013	F1	50	43
B21	NC-2013	F1	50	43
D25	NC-2013	F1	50	40
C52	NC-2013	F1	50	39
E009	NC-2013	F1	50	41
C95	NC-2013	F1	14	2
C100	NC-2013	F1	50	48
D148	NC-2013	F1	50	45
B98	NC-2013	F1	50	42
D65	NC-2013	F1	50	36
B72	NC-2013	F1	50	42
B11	NC-2013	F1	13	7
C9	NC-2013	F1	50	43
C43	NC-2013	F1	50	47
D184	NC-2013	F1	50	43
E12	NC-2013	F1	50	41
C29	NC-2013	F1	50	41
B95	NC-2013	F1	50	48
D87	NC-2013	F1	50	46
D57	NC-2013	F1	50	44
C38	NC-2013	F1	50	32
B22	NC-2013	F1	50	34
C2	NC-2013	F1	50	43
D332	NC-2013	F1	50	48
D162	NC-2013	F1	50	44
D24	NC-2013	F1	50	40
D79	NC-2013	F1	50	38
C12	NC-2013	F1	50	44
D202	NC-2013	F1	50	42
B81	NC-2013	F1	50	38
C90	NC-2013	F1	50	45
C19	NC-2013	F1	50	38
D64	NC-2013	F1	47	31
B93	NC-2013	F1	13	4
B65	NC-2013	F1	12	5
D136	NC-2013	F1	50	27
C64	NC-2013	F1	50	39
D001	NC-2013	F1	50	39

D50	NC-2013	F1	50	19
D155	NC-2013	F1	50	29
D308	NC-2013	F1	4	0
D33	NC-2013	F1	42	27
D123	NC-2013	F1	2	1
D196	NC-2013	F1	27	12
B189	NC-2013	F1	2	0
D83	NC-2013	F1	50	47
C106	NC-2013	F1	50	44
B34	NC-2013	F1	50	43
C13	NC-2013	F1	50	38
B16	NC-2013	F1	5	3
D112	NC-2013	F1	50	36
D95	NC-2013	F1	50	38
B32	NC-2013	F1	50	29
C77	NC-2013	F1	13	8
B94	NC-2013	F1	50	39
B83	NC-2013	F1	50	27
B56	NC-2013	F1	26	15
D006	NC-2013	F1	50	40
E27	NC-2013	F1	1	0
B47	NC-2013	F1	26	16
D335	NC-2013	F1	4	2
D311	NC-2013	F1	25	18
B0	NC-2013	F1	5	2
D318	NC-2013	F1	12	9
D314	NC-2013	F1	3	1

Container #	Cohort	Generation	# Larva in	# Live adults
			Total:	Total live:
D191	NC-F1-2012	F2	48	28
D337	NC-F1-2012	F2	20	12
D145	NC-F1-2012	F2	41	41
D163	NC-F1-2012	F2	49	32
B20	NC-F1-2012	F2	20	20
C54	NC-F1-2012	F2	50	46
C15	NC-F1-2012	F2	50	49
C87	NC-F1-2012	F2	50	38
E23	NC-F1-2012	F2	11	9
D323	NC-F1-2012	F2	50	43
D319	NC-F1-2012	F2	50	47
C42	NC-F1-2012	F2	50	43
D59	NC-F1-2012	F2	50	38
C80	NC-F1-2012	F2	15	12
D128	NC-F1-2012	F2	50	43
C100	NC-F1-2012	F2	50	40
D105	NC-F1-2012	F2	50	27
D179	NC-F1-2012	F2	50	29
D152B	NC-F1-2012	F2	50	34
B104	NC-F1-2012	F2	50	33
D329	NC-F1-2012	F2	50	39
D117	NC-F1-2012	F2	50	40
C76	NC-F1-2012	F2	2	0
C46	NC-F1-2012	F2	12	5
C50	NC-F1-2012	F2	50	38
E34	NC-F1-2012	F2	50	41
E21	NC-F1-2012	F2	50	39
E002	NC-F1-2012	F2	23	13
E20	NC-F1-2012	F2	50	39
E19	NC-F1-2012	F2	50	35
D45	NC-F1-2012	F2	8	5
D44	NC-F1-2012	F2	50	31
D91	NC-F1-2012	F2	50	32
D153	NC-F1-2012	F2	34	14
D166	NC-F1-2012	F2	50	36
B4	NC-F1-2012	F2	50	21
B86	NC-F1-2012	F2	50	36
C26	NC-F1-2012	F2	50	45
D316	NC-F1-2012	F2	50	36

C98	NC-F1-2012	F2	6	3
E10	NC-F1-2012	F2	50	36
E11	NC-F1-2012	F2	50	34
E26	NC-F1-2012	F2	9	5
B61	NC-F1-2012	F2	50	39
C4	NC-F1-2012	F2	50	39
C51	NC-F1-2012	F2	20	11
E13	NC-F1-2012	F2	50	26
C55	NC-F1-2012	F2	16	3
E005	NC-F1-2012	F2	32	22
B001	NC-F1-2012	F2	50	29
D176	NC-F1-2012	F2	4	1
C68	NC-F1-2012	F2	50	34
D74	NC-F1-2012	F2	50	40
B118	NC-F1-2012	F2	10	6
C48	NC-F1-2012	F2	7	1
B9	NC-F1-2012	F2	50	22
B87	NC-F1-2012	F2	47	39
D307	NC-F1-2012	F2	1	0
C112	NC-F1-2012	F2	6	2
B91	NC-F1-2012	F2	7	5
D28	NC-F1-2012	F2	50	41
B73	NC-F1-2012	F2	20	13
D99	NC-F1-2012	F2	3	2
D305	NC-F1-2012	F2	50	19
D309	NC-F1-2012	F2	16	7
D187	NC-F1-2012	F2	50	36
B108	NC-F1-2012	F2	41	26
C8	NC-F1-2012	F2	50	41
B26	NC-F1-2012	F2	1	0
C59	NC-F1-2012	F2	8	4
D192	NC-F1-2012	F2	23	20
D132	NC-F1-2012	F2	6	4

Appendix F Accumulative Data for 2014:

2014 COHORT	Jar	Week	Males	Females	Eggs	Live Larvae	Total Larvae	Larvae per Female
PNW	1	1	2	2	41	25	25	12.5
PNW	1	2	2	2	23	12	13	6.5
PNW	1	3	2	2	24	15	19	9.5
PNW	1	4	2	1	19	30	31	31
PNW	1	5	2	1	24	16	16	16
PNW	1	6	2	1	17	15	17	17
PNW	1	7	2	1	22	10	10	10
PNW	1	8	2	1	10	1	2	2
PNW	2	1	2	2	24	10	12	6
PNW	2	2	1	2	17	17	18	9
PNW	2	3	1	2	46	23	25	12.5
PNW	2	4	1	2	35	19	19	9.5
PNW	2	5	1	2	40	11	12	6
PNW	2	6	1	2	16	7	7	3.5
PNW	2	7	1	2	16	8	8	4
PNW	2	8	1	1	11	0	0	0
PNW	3	1	2	2	45	31	31	15.5
PNW	3	2	2	2	32	22	14	7
PNW	3	3	2	2	20	15	17	8.5
PNW	3	4	2	2	29	13	14	7
PNW	3	5	2	2	22	12	14	7
PNW	3	6	2	2	12	4	6	3
PNW	3	7	1	2	26	7	10	5
PNW	3	8	1	2	8	2	3	1.5
PNW	4	1	2	2	27	10	10	5
PNW	4	2	2	2	35	14	17	8.5
PNW	4	3	1	2	37	27	28	14
PNW	4	4	1	2	29	6	6	3
PNW	4	5	1	2	40	19	20	10
PNW	4	6	1	2	22	12	13	6.5
PNW	4	7	1	2	14	6	6	3
PNW	4	8	1	1	15	1	1	1
PNW	5	1	2	2	40	18	19	9.5
PNW	5	2	2	2	27	19	19	9.5
PNW	5	3	2	2	37	8	8	4
PNW	5	4	1	2	24	10	11	5.5
PNW	5	5	1	2	19	8	8	4

PNW	5	6	1	1	20	3	3	3
PNW	5	7	1	1	24	3	3	3
PNW	5	8	1	1	6	2	2	2
PNW	6	1	2	2	38	14	16	8
PNW	6	2	2	2	40	16	16	8
PNW	6	3	2	2	30	16	16	8
PNW	6	4	2	2	28	24	24	12
PNW	6	5	2	2	24	17	17	8.5
PNW	6	6	2	2	24	13	14	7
PNW	6	7	2	2	30	11	11	5.5
PNW	6	8	2	2	41	6	6	3
PNW	7	1	2	2	39	21	22	11
PNW	7	2	2	2	31	22	22	11
PNW	7	3	2	2	32	23	26	13
PNW	7	4	2	2	29	25	25	12.5
PNW	7	5	2	2	36	24	25	12.5
PNW	7	6	2	2	21	20	20	10
PNW	7	7	2	2	24	13	13	6.5
PNW	7	8	2	2	23	3	3	1.5
PNW	8	1	2	2	34	14	14	7
PNW	8	2	2	2	22	13	13	6.5
PNW	8	3	2	2	43	8	9	4.5
PNW	8	4	2	2	32	19	19	9.5
PNW	8	5	2	2	9	5	6	3
PNW	8	6	2	2	10	4	4	2
PNW	8	7	2	2	26	7	7	3.5
PNW	8	8	2	2	4	1	1	0.5

2014 COHORT	Jar	Week	Males	Females	Eggs	Live Larvae	Total Larvae	Larvae per Female
PNW-F1	1	1	2	2	14	6	6	3
PNW-F1	1	2	2	2	17	3	3	1.5
PNW-F1	1	3	2	2	7	0	0	0
PNW-F1	1	4	2	2	3	4	4	2
PNW-F1	1	5	2	2	4	1	3	1.5
PNW-F1	1	6	2	2	3	1	1	0.5
PNW-F1	1	7	2	2	3	1	1	0.5
PNW-F1	1	8	2	2	1	1	1	0.5
PNW-F1	2	1	2	2	20	16	16	8
PNW-F1	2	2	2	2	18	19	36	18
PNW-F1	2	3	2	2	29	13	13	6.5
PNW-F1	2	4	2	2	27	6	7	3.5
PNW-F1	2	5	2	2	17	14	14	7
PNW-F1	2	6	2	2	14	7	7	3.5
PNW-F1	2	7	2	2	12	9	9	4.5
PNW-F1	2	8	2	2	9	3	3	1.5
PNW-F1	3	1	2	2	19	10	10	5
PNW-F1	3	2	2	2	17	5	5	2.5
PNW-F1	3	3	2	2	13	13	14	7
PNW-F1	3	4	2	2	3	4	4	2
PNW-F1	3	5	2	2	8	7	8	4
PNW-F1	3	6	2	2	8	6	6	3
PNW-F1	3	7	2	2	14	6	7	3.5
PNW-F1	3	8	2	2	13	9	9	4.5
PNW-F1	4	1	2	2	24	13	13	6.5
PNW-F1	4	2	2	2	22	16	16	8
PNW-F1	4	3	2	2	27	17	20	10
PNW-F1	4	4	2	2	15	8	8	4
PNW-F1	4	5	2	2	25	13	13	6.5
PNW-F1	4	6	2	2	17	2	2	1
PNW-F1	4	7	2	2	14	4	4	2
PNW-F1	4	8	1	2	9	3	3	1.5
PNW-F1	5	1	2	2	16	8	8	4
PNW-F1	5	2	2	2	15	11	13	6.5
PNW-F1	5	3	2	2	8	12	13	6.5
PNW-F1	5	4	2	2	10	9	9	4.5
PNW-F1	5	5	2	2	4	7	7	3.5
PNW-F1	5	6	2	2	4	6	6	3
PNW-F1	5	7	2	2	3	1	1	0.5

PNW-F1	5	8	2	2	6	5	5	2.5
PNW-F1	6	1	2	2	30	16	17	8.5
PNW-F1	6	2	2	2	29	21	22	11
PNW-F1	6	3	2	2	21	26	26	13
PNW-F1	6	4	2	2	20	19	19	9.5
PNW-F1	6	5	2	2	13	3	4	2
PNW-F1	6	6	2	2	18	6	6	3
PNW-F1	6	7	2	2	14	8	8	4
PNW-F1	6	8	2	2	21	7	7	3.5
PNW-F1	7	1	2	2	17	8	8	4
PNW-F1	7	2	2	2	15	3	4	2
PNW-F1	7	3	2	2	24	3	5	2.5
PNW-F1	7	4	2	2	14	9	9	4.5
PNW-F1	7	5	2	2	13	8	8	4
PNW-F1	7	6	2	2	17	9	9	4.5
PNW-F1	7	7	2	2	15	9	10	5
PNW-F1	7	8	2	2	13	4	4	2
PNW-F1	8	1	2	2	24	11	11	5.5
PNW-F1	8	2	2	2	16	7	7	3.5
PNW-F1	8	3	2	2	31	11	12	6
PNW-F1	8	4	2	2	13	7	8	4
PNW-F1	8	5	2	2	17	10	11	5.5
PNW-F1	8	6	2	2	19	10	10	5
PNW-F1	8	7	1	2	30	16	16	8
PNW-F1	8	8	1	2	20	7	7	3.5

2014 COHORT	Jar	Week	Males	Females	Eggs	Live Larvae	Total Larvae	Larvae per Female
NC	1	1	2	2	29	13	13	6.5
NC	1	2	2	2	20	13	14	7
NC	1	3	2	2	12	9	11	5.5
NC	1	4	2	2	12	11	11	5.5
NC	1	5	2	1	9	5	5	5
NC	1	6	2	1	4	1	1	1
NC	1	7	2	1	1	1	1	1
NC	1	8	2	1	0	0	0	0
NC	2	1	2	2	21	23	23	11.5
NC	2	2	2	2	29	14	14	7
NC	2	3	2	2	43	16	17	8.5
NC	2	4	2	2	34	19	20	10
NC	2	5	2	2	36	26	31	15.5
NC	2	6	2	2	24	9	9	4.5
NC	2	7	2	2	30	9	9	4.5
NC	2	8	2	2	27	13	13	6.5
NC	3	1	2	2	38	8	8	4
NC	3	2	2	2	23	13	13	6.5
NC	3	3	2	2	21	10	14	7
NC	3	4	2	2	26	16	19	9.5
NC	3	5	2	2	21	21	23	11.5
NC	3	6	2	2	30	10	10	5
NC	3	7	2	2	19	9	9	4.5
NC	3	8	2	2	8	0	0	0
NC	4	1	2	2	27	13	14	7
NC	4	2	2	2	42	17	22	11
NC	4	3	2	2	22	10	11	5.5
NC	4	4	2	2	14	7	10	5
NC	4	5	2	2	19	11	11	5.5
NC	4	6	2	2	11	4	4	2
NC	4	7	2	2	21	9	9	4.5
NC	4	8	2	2	18	2	2	1
NC	5	1	2	2	27	2	3	1.5
NC	5	2	2	2	40	20	21	10.5
NC	5	3	2	2	33	7	8	4
NC	5	4	3	1	10	13	13	13
NC	5	5	3	1	9	13	16	16
NC	5	6	3	1	11	4	4	4
NC	5	7	3	1	4	3	3	3

NC	5	8	2	1	8	3	3	3
NC	6	1	2	2	34	13	14	7
NC	6	2	2	2	32	26	27	13.5
NC	6	3	2	2	14	12	14	7
NC	6	4	1	1	18	12	13	13
NC	6	5	1	1	4	11	11	11
NC	6	6	1	1	13	6	6	6
NC	6	7	1	1	15	4	4	4
NC	6	8	1	1	11	7	7	7
NC	7	1	2	2	21	9	9	4.5
NC	7	2	2	2	23	17	20	10
NC	7	3	2	2	27	14	15	7.5
NC	7	4	2	2	14	18	18	9
NC	7	5	2	2	17	20	20	10
NC	7	6	2	2	23	9	9	4.5
NC	7	7	2	2	19	9	9	4.5
NC	7	8	2	2	7	2	3	1.5
NC	8	1	2	2	21	4	5	2.5
NC	8	2	2	2	16	22	22	11
NC	8	3	2	2	23	14	17	8.5
NC	8	4	2	2	22	9	12	6
NC	8	5	2	2	8	9	10	5
NC	8	6	2	2	25	7	8	4
NC	8	7	2	2	20	8	8	4
NC	8	8	2	2	17	5	6	3

2014 COHORT	Jar	Week	Males	Females	Eggs	Live Larvae	Total Larvae	Larvae per Female
NC-F1	1	1	2	2	22	6	6	3
NC-F1	1	2	2	2	19	11	11	5.5
NC-F1	1	3	2	2	23	11	12	6
NC-F1	1	4	2	2	16	6	6	3
NC-F1	1	5	2	2	21	5	6	3
NC-F1	1	6	2	2	11	5	5	2.5
NC-F1	1	7	2	2	4	1	1	0.5
NC-F1	1	8	2	2	5	4	4	2
NC-F1	2	1	2	2	28	17	17	8.5
NC-F1	2	2	2	2	20	30	32	16
NC-F1	2	3	2	2	23	12	15	7.5
NC-F1	2	4	2	2	21	11	12	6
NC-F1	2	5	2	2	19	9	9	4.5
NC-F1	2	6	1	2	23	7	7	3.5
NC-F1	2	7	1	2	15	4	4	2
NC-F1	2	8	1	2	8	1	1	0.5
NC-F1	3	1	2	2	4	1	1	0.5
NC-F1	3	2	2	2	3	2	2	1
NC-F1	3	3	2	2	4	0	0	0
NC-F1	3	4	2	2	1	0	1	0.5
NC-F1	3	5	2	2	2	1	1	0.5
NC-F1	3	6	2	2	0	0	0	0
NC-F1	3	7	2	2	7	2	2	1
NC-F1	3	8	2	2	4	2	2	1
NC-F1	4	1	2	2	24	2	2	1
NC-F1	4	2	2	2	17	12	13	6.5
NC-F1	4	3	2	2	16	3	5	2.5
NC-F1	4	4	2	2	14	8	10	5
NC-F1	4	5	2	2	17	10	10	5
NC-F1	4	6	2	2	18	10	10	5
NC-F1	4	7	2	2	10	3	3	1.5
NC-F1	4	8	2	2	9	3	3	1.5
NC-F1	5	1	2	2	21	8	8	4
NC-F1	5	2	2	2	20	5	5	2.5
NC-F1	5	3	2	2	11	9	9	4.5
NC-F1	5	4	1	2	12	9	12	6
NC-F1	5	5	1	2	7	4	4	2
NC-F1	5	6	1	2	4	2	2	1
NC-F1	5	7	1	2	5	0	0	0

NC-F1	5	8	1	2	2	3	3	1.5
NC-F1	6	1	2	2	22	17	17	8.5
NC-F1	6	2	2	2	17	14	14	7
NC-F1	6	3	2	2	18	17	17	8.5
NC-F1	6	4	2	2	17	5	6	3
NC-F1	6	5	2	2	14	6	6	3
NC-F1	6	6	2	2	15	9	9	4.5
NC-F1	6	7	2	2	15	0	0	0
NC-F1	6	8	2	2	10	4	4	2
NC-F1	7	1	2	2	9	8	9	4.5
NC-F1	7	2	2	2	16	14	14	7
NC-F1	7	3	2	2	9	7	7	3.5
NC-F1	7	4	2	2	9	6	7	3.5
NC-F1	7	5	2	2	11	4	4	2
NC-F1	7	6	2	2	14	6	6	3
NC-F1	7	7	2	2	12	5	5	2.5
NC-F1	7	8	2	2	8	3	3	1.5
NC-F1	8	1	2	2	14	8	8	4
NC-F1	8	2	2	2	16	7	8	4
NC-F1	8	3	2	2	16	5	5	2.5
NC-F1	8	4	2	2	12	4	6	3
NC-F1	8	5	2	2	8	11	11	5.5
NC-F1	8	6	1	2	21	7	7	3.5
NC-F1	8	7	1	2	14	6	6	3
NC-F1	8	8	0	2	3	1	1	0.5

REFERENCES

Arsenault, A. L. (2013). *Behavioral Ecology and Genetics of Potential Natural Enemies of Hemlock Woolly Adelgid* (Master's thesis, The University of Vermont).

Cheah, C., Montgomery, M., Salom, S., Parker, B., Skinner, M., & Costa, S. (2004). Biological control of hemlock woolly adelgid. *USDA, Forest Service*, FHTET-2004-04.

Cohen, A. C., & Cheah, C. (2011). Development of Artificial Diets for Predators of Hemlock Woolly Adelgids. *Implementation and Status of Biological control of the hemlock woolly adelgid*, 148.

Dilling, C., Lambdin, P., Grant, J., & Buck, L. (2007). Insect guild structure associated with eastern hemlock in the southern Appalachians. *Environmental entomology*, *36*(6), 1408-1414.

Eschtruth, A. K., Cleavitt, N. L., Battles, J. J., Evans, R. A., & Fahey, T. J. (2006). Vegetation dynamics in declining eastern hemlock stands: 9 years of forest response to hemlock woolly adelgid infestation. *Canadian Journal of Forest Research*, *36*(6), 1435-1450.

Fidgen, J. G., McClellan, Q. C., & Salom, S. M. (2002). Efficacy and residual activity of two systemic insecticides for control of hemlock woolly adelgid on young eastern hemlocks. In *Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium* (329-333). Rutgers University.

Goodman, R. M., & Lancaster, K. (1990). Tsuga canadensis–Eastern hemlock in Silvics of North America: Volume 1 Conifers. *USDA Forest Service Agriculture Handbook*.

Havill, N. P., Montgomery, M. E., Yu, G., Shiyake, S., & Caccone, A. (2006). Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America*, *99*(2), 195-203.

Havill, N. P., & Foottit, R. G. (2007). Biology and evolution of Adelgidae. *Annual Review Entomology*, *52*, 325-349.

Havill, N. P., & Montgomery, M. E. (2008). The role of arboreta in studying the evolution of host resistance to the hemlock woolly adelgid. *Arnoldia*, *65*(3), 1-9.

Havill, N. P., Davis, G., Mausel, D. L., Klein, J., McDonald, R., Jones, C., Fischer, M., Salom, S., & Caccone, C. (2012). Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. *Biological Control, 63*, 359-369.

Havill, N.P., Vieira, L.C., & Salom, S.M. (2014). Biology and control of hemlock wooly adelgid. United States Department of Agriculture.

Klein, J. L., Caccone, A., & Havill, N. P. (2010). Polymorphic microsatellite loci for Laricobius nigrinus and L. rubidus (Coleoptera: Derodontidae), predators of the hemlock woolly adelgid.

Lamb, A. (n.d.) *Laricobius nigrinus* egg (yellow) amid wool and red eggs of HWA [Photograph]. Retrieved from http://www.Forestryimages.org

Lamb, A. B., Salom, S. M., & Kok, L. T. (2005). Guidelines for rearing Laricobius nigrinus Fender. In *Proceedings: Third Symposium on Hemlock Woolly Adelgid in the Eastern United States*, 309-318.

Lamb, A. B., Salom, S. M., & Kok, L. T. (2007). Factors influencing aestivation in Laricobius nigrinus (Coleoptera: Derodontidae), a predator of Adelges tsugae (Hemiptera: Adelgidae). *The Canadian Entomologist*, *139*(04), 576-586.

Lawrence, J.F. (1989). A Catalog of the Coleoptera of America North of Mexico, Family: Derodontidae. *USDA Agriculture Handbook,* 529-565.

Martin, K. L., & Goebel, P. C. (2012). Decline in riparian Tsuga canadensis forests of the central Appalachians across an Adelges tsugae invasion consequence. *The Journal of the Torrey Botanical Society*, *139*(4), 367-378.

Mausel, D. L., Salom, S. M., Kok, L. T., & Fidgen, J. G. (2008). Propagation, synchrony, and impact of introduced and native Laricobius spp. (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. *Environmental entomology*, *37*(6), 1498-1507.

Mausel, D. L., Salom, S. M., Kok, L. T., & Davis, G. A. (2010). Establishment of the hemlock woolly adelgid predator, Laricobius nigrinus (Coleoptera: Derodontidae), in the eastern United States. *Environmental entomology*, *39*(2), 440-448.

McClure, M. S. (1987). Biology and control of hemlock woolly adelgid. *Bulletin of the Connecticut Agricultural Experiment Station*, *851*(9).

McClure, M. S. (1989). Evidence of a polymorphic life cycle in the hemlock woolly adelgid, Adelges tsugae (Homoptera: Adelgidae). *Annals of the Entomological Society of America*, *82*(1), 50-54.

McClure, M. S. (1996). Biology of Adelges tsugae and its potential for spread in the northeastern United States. In S. M. Salom, T. C. Tigner, & R. C. Reardon (Eds.), *Proceedings of the first hemlock woolly adelgid review* (16-25). Charlottesville, Virginia: Forest Health Technology Enterprise Team, USDA Forest Service.

McClure, M. S., & Cheah, C. A. J. (1999). Reshaping the ecology of invading populations of hemlock woolly adelgid, Adelges tsugae (Homoptera: Adelgidae), in eastern North America. *Biological Invasions*, *1*(2-3), 247-254.

Reay, R. S. (2000). Management of eastern hemlock for deer wintering areas. In *Proceedings: Symposium on sustainable management of hemlock ecosystems in eastern North America. USDA Forest Service, Northeastern Research Station, GTR-NE-267* (pp. 144-147).

Ross, R. M., Bennett, R. M., Snyder, C. D., Young, J. A., Smith, D. R., & Lemarie, D. P. (2003). Influence of eastern hemlock (Tsuga canadensis L.) on fish community structure and function in headwater streams of the Delaware River basin. *Ecology of Freshwater Fish*, *12*(1), 60-65.

Salom, S. M., Kok, L. T., Lamb, A. B., & Jubb, C. (2012). Laboratory rearing of Laricobius nigrinus (Coleoptera: Derodontidae): A predator of the hemlock woolly adelgid (Hemiptera: Adelgidae). *Psyche: A Journal of Entomology*, *2012*.

Shepherd, W. P., Montgomery, M. E., Sullivan, B. T., & Mayfield, A. E. (2014). Novel method for determining sex of live adult Laricobius nigrinus Coleoptera: Derodontidae). *The Canadian Entomologist*, 1-4.

Shields, K. S., Young, R. F., & Berlyn, G. P. (1995). Hemlock woolly adelgid feeding mechanisms. *Proceedings of the First Hemlock Woolly Adelgid Review, Charlottesville, VA*, 96-10.

Stoetzel, M. B. (2002). History of the introduction of Adelges tsugae based on voucher specimens in the Smithsonian Institute National Collection of Insects. In *Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium*, 12.

Taylor, R. J. (1993). Sections on Picea and Tsuga. Flora of North America Editorial Committee (eds.): *Flora of North America North of Mexico, Vol. 2*. Oxford University Press.

Tingley, M. W., Orwig, D. A., Field, R., & Motzkin, G. (2002). Avian response to removal of a forest dominant: consequences of hemlock woolly adelgid infestations. *Journal of Biogeography*, *29*(10-11), 1505-1516.

United States Department of Agriculture Natural Resources Conservation Service (n.d.). *Tsuga caroliniana Eglem. Carolina hemlock distribution map, USDA Forest Service* [Map]. Retrieved from http://plants.usda.gov/core/profile?symbol=TSCA2

United States Department of Agriculture Forest Service. (2013). *Hemlock woolly adelgid, forest health protection, usda forest service* [Map]. Retrieved from http://na.fs.fed.us/fhp/hwa/maps/distribution.shtm

Vieira, L.C., Salom, S.M., & Kok, L.T., (2012). Functional and numerical response of Laricobius spp. predators (Coleoptera: Derodontidae) on hemlock woolly adelgid, Adelges tsugae (Hemiptera: Adelgidae). *Biological Control, 61,* 47-54.

Wallin, K. F., Latty, T. M., & Ross, D. W. (2011). Orientation behavior of the predator Laricobius nigrinus (Coleoptera: Derodontidae) to hemlock woolly adelgid and host tree odors in a multi-chambered olfactometer. *Environmental entomology*, *40*(4), 864-872.

Webb, R. E., Frank, J. R., & Raupp, M. J. (2003). Eastern Hemlock Recovery From Hemlock Wooly Adelgid Damage Following Imidaclopid Therapy. *Journal of Arboriculture*, *29*(5), 298-302.

Young, R. F., Shields, K. S., & Berlyn, G. P. (1995). Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America*, *88*(6), 827-835.

Zilahi-Balogh, G. M. (2001). *Biology of Laricobius nigrinus Fender (Coleoptera: Derodontidae) and its potential as a biological control agent of the hemlock woolly adelgid, Adelges tsugae Annand (Homoptera: Adelgidae) in the eastern United States* (Doctoral dissertation, Virginia Polytechnic Institute and State University).

Zilahi-Balogh, G. M. G., Kok, L. T., & Salom, S. M. (2002). Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). *Biological Control*, *24*(2), 192-198.

Zilahi-Balogh, G. M. G., Salom, S. M., & Kok, L. T. (2003). Development and reproductive biology of Laricobius nigrinus, a potential biological control agent of Adelges tsugae. *Biocontrol*, *48*(3), 293-306.